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

On the cover: The chemical structure of diacetyl

Criteria for a Recommended Standard

Occupational Exposure to Diacetyl and 2,3-Pentanedione

McKernan LT, Niemeier RT, Kreiss K, Hubbs A, Park R, Dankovic D, Dunn KH, Parker J, Fedan K, Streicher R, Fedan J, Garcia A, Whittaker C, Gilbert S, Nourian F, Galloway E, Smith R, Lentz TJ, Hirst D, Topmiller J, Curwin B

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

Disclaimer

Mention of any company or product does not constitute endorsement by the National Institute for Occupational Safety and Health (NIOSH). In addition, citations of websites external to NIOSH do not constitute NIOSH endorsement of the sponsoring organizations or their programs or products. Furthermore, NIOSH is not responsible for the content of these websites. The content of this document is intended for occupational exposures and does not address consumer exposures.

Ordering Information

This document is in the public domain and may be freely copied or reprinted. To receive NIOSH documents or other information about occupational safety and health topics, contact NIOSH at

Telephone: 1-800-CDC-INFO (1-800-232-4636)
TTY: 1-888-232-6348
E-mail: cdcinfo@cdc.gov

or visit the NIOSH website at www.cdc.gov/niosh.

For a monthly update on news at NIOSH, subscribe to *NIOSH eNews* by visiting www.cdc.gov/niosh/eNews.

Suggested Citation

NIOSH [2016]. Criteria for a recommended standard: occupational exposure to diacetyl and 2,3-pentanedione. By McKernan LT, Niemeier RT, Kreiss K, Hubbs A, Park R, Dankovic D, Dunn KH, Parker J, Fedan K, Streicher R, Fedan J, Garcia A, Whittaker C, Gilbert S, Nourian F, Galloway E, Smith R, Lentz TJ, Hirst D, Topmiller J, Curwin B. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2016-111.

DHHS (NIOSH) Publication No. 2016-111

October 2016

Foreword

When the U.S. Congress passed the Occupational Safety and Health Act of 1970 (Public Law 91-596), it established the National Institute for Occupational Safety and Health. Through the Act, Congress charged NIOSH with recommending occupational safety and health standards and describing exposure levels that are safe for various periods of employment, including but not limited to the exposures at which no employee will suffer diminished health, functional capacity, or life expectancy because of his or her work experience.

Criteria documents contain a critical review of the scientific and technical information about the prevalence of hazards, the existence of safety and health risks, and the adequacy of control methods. By means of criteria documents, NIOSH communicates these recommended standards to regulatory agencies, including the Occupational Safety and Health Administration, health professionals in academic institutions, industry, organized labor, public interest groups, and others in the occupational safety and health community.

This criteria document is derived from the NIOSH evaluation of critical health effects studies of occupational exposure to diacetyl and 2,3-pentanedione. It provides recommendations for controlling workplace exposures including recommended exposure limits derived by using current quantitative risk assessment methodology on human and animal health effects data.

Using cross-sectional pulmonary function data from diacetyl-exposed employees, NIOSH conducted assessments to determine the exposure-response relationship and to 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 analysis, NIOSH recommends keeping exposure to diacetyl below a concentration of 5 parts per billion as a time-weighted average during a 40-hour work week. To further protect against effects of short-term exposures, NIOSH recommends a short-term exposure limit for diacetyl of 25 parts per billion for a 15-minute time period.

In many operations, 2,3-pentanedione is being used to substitute for diacetyl. Published toxicological studies indicate that 2,3-pentanedione exposure can cause damage similar to that caused by diacetyl in laboratory studies. Therefore, NIOSH recommends keeping occupational exposure to 2,3-pentanedione below a level comparable to the level recommended for diacetyl. However, the recommended sampling and analytical method can only reliably quantify it to 9.3 parts per billion in an 8-hour sample. NIOSH also recommends a short-term exposure limit for 2,3-pentanedione of 31 parts per billion during a 15-minute period.

Engineering and work practices are available to control diacetyl and 2,3-pentanedione exposures below the recommended exposure limits. A hierarchy of controls including elimination, substitution, engineering controls, administrative controls, and the use of personal protective equipment should be followed to control workplace exposures.

NIOSH urges employers to disseminate this information to employees and customers. NIOSH also requests that professional and trade associations and labor organizations inform their members about the hazards of occupational exposure to these flavoring compounds.

NIOSH appreciates the time and effort of the expert peer, stakeholder, and public reviewers whose comments and input strengthened this document.

John Howard, MD
Director, National Institute for
Occupational Safety and Health
Centers for Disease Control and Prevention

Executive Summary

Diacetyl and its substitute, 2,3-pentanedione, are widely used flavoring compounds. There have been extensive reports of serious respiratory disease and decreased lung function in employees exposed to diacetyl. The NIOSH objective in establishing recommended exposure limits (RELs) for diacetyl and 2,3-pentanedione is to reduce the risk of respiratory impairment (decreased lung function) and the severe irreversible lung disease obliterative bronchiolitis associated with occupational exposure to these compounds. In this disease the smallest airways in the lungs, the bronchioles, become scarred and constricted, blocking the movement of air. 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 employees. 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; however, additional considerations include sampling and analytical feasibility and the achievability of engineering controls.

Diacetyl is used extensively in the food flavoring and production industries, and occupational exposure to this substance has been associated with severe respiratory impairment and the disease obliterative bronchiolitis. 2,3-Pentanedione, which has been used as a substitute for diacetyl, is also of concern because of structural similarities with diacetyl and because animal studies show similar toxicity for the respiratory tract [Hubbs et al. 2012; Morgan et al. 2012; Morgan et al. 2016].

The first observation of obliterative bronchiolitis in a food production employee may have occurred in 1985 in a facility where diacetyl was listed among ingredients used in making flavorings for the baking industry [NIOSH 1985]. The link between exposure to diacetyl and lower pulmonary function was confirmed in the early 2000s, and research further showed that diacetyl exposure leads to a decrease in pulmonary function [Kreiss et al. 2002]. Occupational exposures to diacetyl have been assessed in a variety of food production and flavoring facilities [Kanwal et al. 2006; Martyny et al. 2008; NIOSH 2003a, b, 2004a, b, 2006, 2007, 2008a, b, 2009, 2011].

Another compound, acetoin, was present along with diacetyl in many of the workplaces where obliterative bronchiolitis occurred in employees who made or used diacetyl [Kullman et al. 2005; van Rooy et al. 2007]. However, current data indicate that acetoin is considerably less hazardous than diacetyl and it does not have the reactive α-dicarbonyl group, which has been implicated in the toxicity of diacetyl and 2,3-pentanedione [Hubbs et al. 2016; National Toxicology Program 2015; Zaccone et al. 2013].

Mean diacetyl air concentrations measured at the first microwave popcorn facility where obliterative bronchiolitis was reported were highest in the mixing room (57.2 parts per million [ppm]), followed by the packaging area (2.8 ppm) [Kanwal et al. 2011]. Mean personal diacetyl air concentrations at five other microwave popcorn plants were lower: 0.023 to 1.16 ppm in the mixing room and 0.35 to 1.33 ppm in the packaging rooms/areas [Kanwal et al. 2006]. Mean full-shift diacetyl air concentrations measured

at flavor manufacturing facilities ranged from 0.07 ppm to 2.73 ppm [Kanwal et al. 2006; Martyny et al. 2008; NIOSH 2003a, b, 2004a, b, 2006, 2007, 2008a, b, 2009, 2011].

In addition to cases consistent with obliterative bronchiolitis in flavoring manufacturing, diacetyl manufacturing, and microwave popcorn production, case reports have surfaced in other industries in which flavorings are introduced. In cookie manufacturing with artificial butter flavoring in Brazil, four cases of bronchiolitis were described in young men, aged 24 to 27 years, who had worked between 1 and 3 years handling flavorings in preparation of cookie dough [Cavalcanti et al. 2012]. In a coffee production plant, two cases have biopsy confirmation of obliterative bronchiolitis among employees with artificial flavorings exposure in the production of roasted coffee beans and ground coffee [CDC 2013]. In 2012, NIOSH conducted a health hazard evaluation (HHE) involving 75 current employees (88% participation) [Bailey et al. 2015]. Excluding the five sentinel former employees (all never-smokers under age 42), standardized morbidity ratios were elevated 1.6-fold for shortness of breath and 2.7-fold for obstructive spirometric abnormalities.

Investigations of severe lung disease consistent with obliterative bronchiolitis among diacetyl-exposed employees presented in Chapter 3 have provided substantial evidence of a causal relationship between diacetyl exposure and development of this disease. These findings in conjunction with laboratory experiments providing biological plausibility, meet the standard criteria used to determine causation: that an exposure is the likely cause of specific health effects [Gordis 1996; Hill 1965].

NIOSH has reviewed the literature on diacetyl toxicology and exposures in the workplace and subsequently conducted a quantitative risk assessment. The quantitative risk assessment used to derive the REL was based solely on human (employee) data, but the results were informed and corroborated by animal risk assessments. On the basis of a quantitative risk assessment of data collected in a series of NIOSH health hazard investigations (full description in Chapter 5), NIOSH has concluded that employee exposure to diacetyl is associated with a reduction in lung function. Specifically, a statistically significant exposure-associated reduction in the forced expiratory volume in one second/forced vital capacity (FEV_1/FVC) ratio and percent predicted FEV_1 ($ppFEV_1$) and an exposure-associated estimated incidence of symptomatic 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 5.35). Lifetime risks in the range of 1:1,000 corresponded to working lifetime diacetyl exposure of approximately 5 parts per billion (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 a REL at that level.

It should be noted that diacetyl and 2,3-pentanedione are found in cigarette smoke [Fujioka and Shibamoto 2006; Pierce et al. 2014; Polzin et al. 2007] and some flavored e-cigarettes [Allen et al. 2016; Farsalinos et al. 2015]. As extensively discussed in Chapter 3, increased prevalence of airway obstruction and decreased FEV_1 can be identified in smokers who are exposed to diacetyl in comparison to prevalence in smokers in the U.S. population. Most importantly, because diacetyl causes obstructive lung disease and because smoking causes obstructive lung disease, the presence of diacetyl in cigarette smoke in no way diminishes the need to control diacetyl exposures in employees.

NIOSH concludes that the toxicological responses to diacetyl observed in animal studies support the conclusions of the epidemiologically-based risk assessment for diacetyl. Further, the animal-based

risk assessment presented in Chapter 6 corroborates 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 employees exposed to diacetyl in the epidemiologic assessment.

On this basis, NIOSH recommends a REL of 5 ppb for diacetyl as a time-weighted average (TWA) for up to 8 hours/day during a 40-hour workweek. NIOSH has determined that employees 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 1/1,000 excess risk of lung function falling below the lower limit of normal due to diacetyl exposure.

To ensure that employee 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 corrective 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 employee exposures (described in Chapter 10) is helpful to protect employees.

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.

2,3-Pentanedione is used in many operations; it is structurally similar to diacetyl because it is a 5-carbon alpha diketone, whereas diacetyl is a 4-carbon alpha diketone. Published toxicology studies indicate that 2,3-pentanedione exposure can cause damage to the lining of airways similar to that caused by diacetyl in laboratory studies [Hubbs et al. 2012; Morgan et al. 2012; Morgan et al. 2016]. Therefore, NIOSH recommends controlling occupational exposure to 2,3-pentanedione to a level comparable to that recommended for diacetyl. However, analytical limitations allow 2,3-pentanedione to be reliably measured only above 9.3 ppb. This recommended exposure limit is slightly higher than the recommended exposure limit for diacetyl. NIOSH recommends keeping exposure to 2,3-pentanedione below 9.3 ppb in an 8-hour average during a 40-hour work week. NIOSH has estimated that employees exposed to 2,3-pentanedione at this concentration should have a similar risk of decreased pulmonary function as employees exposed to diacetyl. NIOSH also recommends a short-term exposure limit for 2,3-pentanedione of 31 ppb during a 15-minute period.

Research into various flavoring industries has led to the development of engineering controls that may help reduce employee 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. Table 8.2 in Chapter 8 provides a summary of NIOSH-evaluated engineering control efficiencies for the mixing of food flavorings. NIOSH acknowledges that the frequent use of personal protective equipment (PPE), including respirators, may be required for some employees 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.

NIOSH recommends that employers develop and implement comprehensive occupational safety and health programs to protect employees with potential exposure to diacetyl, 2,3-pentanedione, and other potentially hazardous flavoring compounds. This program should include periodic exposure and medical evaluation and monitoring exposure controls and appropriate employee training on potential health effects, respiratory protection, and use of controls. Employers should (1) determine employee exposure to diacetyl, 2,3-pentanedione, and other flavoring compounds used in the workplace; (2) evaluate the effectiveness of work practice and engineering controls; and (3) facilitate the selection of appropriate personal protective equipment. Because diacetyl and 2,3-pentanedione are found in cigarette smoke [Fujioka and Shibamoto 2006; Pierce et al. 2014; Polzin et al. 2007] and e-cigarettes, NIOSH also recommends that all employers make tobacco cessation programs available to employees and have workplaces that are free of tobacco smoking and vaping with flavored nicotine delivery systems [NIOSH 2015].

All permanent, temporary, and contract employees who work in or enter areas where diacetyl, 2,3-pentanedione, or similar flavoring compounds or products that contain these compounds are used or produced should be included in the medical monitoring program. Employees who work in or enter these areas for a total of 40 or more hours per year should be included in the medical monitoring program. Because of the potentially rapid progression and grave consequences of flavoring-related lung disease, it is important that the medical monitoring program director be able to quickly evaluate clinical data and make medical judgments about appropriate diagnostic and therapeutic measures, including medical removal. For this reason, the medical monitoring program director should be a licensed physician with training and experience in identifying and preventing occupational lung disease. The medical program that includes the following:

- good quality spirometry testing for pulmonary function
- medical evaluation for employees found with abnormal spirometry
- removal from exposure pending medical evaluation
- analysis of group medical surveillance and longitudinal spirometry data to assess work-related risk factors on the basis of job, task, area, and other exposure indices

The purpose of this epidemiologic surveillance is to assist monitoring physicians in assessing the likelihood of work-related causes of abnormalities and to prioritize interventions, if needed. Identifying excessive declines in spirometry, even if absolute spirometric values remain within the normal range, offers the best opportunity to intervene before progression to symptomatic impairment and to prevent the development of clinically significant occupational lung disease. The rapid onset and progression of diacetyl-related lung disease requires more frequent medical monitoring evaluations be done than with slowly progressive occupational lung diseases, such as silicosis and coal employees pneumoconiosis. While the focus of this document is on diacetyl and 2,3-pentanedione, NIOSH has concern regarding other volatile and reactive flavorings potentially capable of producing similar toxic effects. Therefore, NIOSH recommends that such exposures be carefully considered and controlled in consultation with workplace safety professionals and the recommendations contained within this criteria document.

References

Allen JG, Flanigan SS, LeBlanc M, Vallarino J, MacNaughton P, Stewart JH, Christiani DC [2016]. Flavoring chemicals in e-cigarettes: diacetyl, 2,3-pentanedione, and acetoin in a sample of 51 products, including fruit-, candy-, and cocktail-flavored e-cigarettes. Environ Health Perspect *124*(6):733–739.

Bailey RL, Cox-Ganser JM, Duling MG, LeBouf RF, Martin SB, Bledsoe TA, Green BJ, Kreiss K [2015]. Respiratory morbidity in a coffee processing workplace with sentinel obliterative bronchiolitis cases. Am J Ind Med *58*(12):1235–1245.

Cavalcanti ZD, Albuquerque Filho AP, Pereira CA, Coletta EN [2012]. Bronchiolitis associated with exposure to artificial butter flavoring in workers at a cookie factory in Brazil. J Bras Pneumol *38*(3):395–399.

CDC [2013]. Obliterative bronchiolitis in workers in a coffee-processing facility – Texas, 2008–2012. MMWR *62*(16):305–307.

Farsalinos KE, Kistler KA, Gillman G, Voudris V [2015]. Evaluation of electronic cigarette liquids and aerosol for the presence of selected inhalation toxins. Nicotine Tob Res *17*(2):168–174.

FEMA (Flavor and Extract Manufacturers Association) [2004]. Respiratory health and safety in the flavor manufacturing workplace. http://www.femaflavor.org/html/public/RespiratoryRpt.pdf. Date accessed: October 2007.

Fujioka K, Shibamoto T [2006]. Determination of toxic carbonyl compounds in cigarette smoke. Environ Toxicol *21*(1):47–54.

Gordis L [1996]. Epidemiology. Philadelphia, PA: W. B. Saunders Company, pp. 176–177.

Hill AB [1965]. The environment and disease: association or causation? Proc R Soc Med *58*:295–300.

Hubbs AF, Cumpston AM, Goldsmith WT, Battelli LA, Kashon ML, Jackson MC, Frazer DG, Fedan JS, Goravanahally MP, Castranova V, Kreiss K, Willard PA, Friend S, Schwegler-Berry D, Fluharty KL, Sriram K [2012]. Respiratory and olfactory cytotoxicity of inhaled 2,3-pentanedione in Sprague-Dawley rats. Am J Pathol *181*(3):829–844.

Hubbs AF, Fluharty KL, Edwards RJ, Barnabei JL, Grantham JT, Palmer SM, Kelly F, Sargent LM, Reynolds SH, Mercer RR, Goravanahally MP, Kashon ML, Honaker JC, Jackson MC, Cumpston AM, Goldsmith WT, McKinney W, Fedan JS, Battelli LA, Munro T, Bucklew-Moyers W, McKinstry K, Schwegler-Berry D, Friend S, Knepp AK, Smith SL, Sriram K [2016]. Accumulation of ubiquitin and sequestosome-1 implicate protein famage in diacetyl-induced cytotoxicity. Am J Pathol *186*(11):2887–2908.

Kanwal R, Kullman G, Fedan K, Kreiss K [2011]. Occupational lung disease risk and exposures to butter flavoring chemicals after implementation of controls at a microwave popcorn plant. Public Health Rep *126*:480–494.

Kanwal R, Kullman G, Piacitelli C, Boylstein R, Sahakian N, Martin S, Fedan K, Kreiss K [2006]. Evaluation of flavorings-related lung disease risk at six microwave popcorn plants. J Occup Environ Med *48*(2):149–157.

Kreiss K, Gomaa A, Kullman G, Fedan K, Simoes EJ, Enright PL [2002]. Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. N Engl J Med *347*(5):330–338.

Kullman G, Boylstein R, Jones W, Piacitelli C, Pendergrass S, Kreiss K [2005]. Characterization of respiratory exposures at a microwave popcorn plant with cases of bronchiolitis obliterans. J Occup Environ Hyg *2*(3):169–178.

Martyny JW, Van Dyke MV, Arbuckle S, Towle M, Rose CS [2008]. Diacetyl exposures in the flavor manufacturing industry. J Occup Environ Hyg *5*(11):679–688.

Morgan DL, Jokinen MP, Price HC, Gwinn WM, Palmer SM, Flake GP [2012]. Bronchial and bronchiolar fibrosis in rats exposed to 2,3-pentanedione vapors: implications for bronchiolitis obliterans in humans. Toxicol Pathol *40*(3):448–465.

National Toxicology Program [2015]. In-Life and Pathology Tables and Curves for Acetoin (513-86-0) http://tools.niehs.nih.gov/cebs3/ntpViews/?investigationNumber=002-01593-0000-0000-0 Date accessed: April 2015.

NIOSH [1985]. Hazard evaluation and technical assistance report: International Bakers Services, Inc, South Bend, IN. By McConnell R, Hartle R. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 1985–0171–1710.

NIOSH [2003a]. Hazard evaluation and technical assistance report: Agrilink Foods Popcorn Plant, Ridgway, IL. By Sahakian N, Choe K, Boylstein R, Schleiff P. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2002–0408–2915.

NIOSH [2003b]. Hazard evaluation and technical assistance report: Nebraska Popcorn, Clearwater, NE. By Kanwal R. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2002–0089.

NIOSH [2004a]. Hazard evaluation and technical assistance report: American Pop Corn Company, Sioux City, IA. By Kanwal R, Boylstein R, Piacitelli C. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2001–0474–2943.

NIOSH [2004b]. Hazard evaluation and technical assistance report: ConAgra Snack Foods, Marion, OH. By Kanwal R, Kullman G. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2003–0112–2949.

NIOSH [2006]. Hazard evaluation and technical assistance report: Gilster-Mary Lee Corporation, Jasper, MO. By Kanwal R, Kullman G, Fedan K, Kreiss K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2000–0401–2991.

NIOSH [2007]. Hazard evaluation and technical assistance report: Carmi Flavor and Fragrance Company, Inc., Commerce, CA. By Kanwal R, Kullman G. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2006–0303–3043.

NIOSH [2008a]. Evaluating occupational exposures and work practices at Agilex Flavors, Inc. formerly Key Essentials, Inc., Rancho Santa Margarita, CA. A technical assistance report to the California/Occupational Safety and Health Administration. By McKernan L, Dunn K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2006–0361–2.

NIOSH [2008b]. Hazard evaluation and technical assistance report: Gold Coast Ingredients, Inc., Commerce, CA. By Bailey R, McKernan L, Dunn K, Sahakian N, Kreiss K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2007–0033–3074.

NIOSH [2009]. Hazard evaluation and technical assistance report: Chr. Hansen, Inc., New Berlin, WI. By Sahakian N, Kullman G, Dunn K, Kanwal R. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2007–0327–3083.

NIOSH [2011]. Hazard evaluation and technical assistance report: Lung function (spirometry) testing in employees at a flavorings manufacturing plant -- Indiana. By Kreiss K, Piacitelli C, Cox-Ganser J. Morgantown, WV: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2008–0155–3131.

NIOSH [2015]. Current intelligence bulletin 67: Promoting health and preventing disease and injury through workplace tobacco policies. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 215–113.

Pierce JS, Abelmann A, Spicer LJ, Adams RE, Finley BL [2014]. Diacetyl and 2,3-pentanedione exposures

associated with cigarette smoking: implications for risk assessment of food and flavoring workers. Crit Rev Toxicol *44*(5):420–425.

Polzin GM, Kosa-Maines RE, Ashley DL, Watson CH [2007]. Analysis of volatile organic compounds in mainstream cigarette smoke. Entviron Sci Technol *41*(4):1297–1302.

van Rooy FG, Rooyackers JM, Prokop M, Houba R, Smit LA, Heederik DJ [2007]. Bronchiolitis obliterans syndrome in chemical workers producing diacetyl for food flavorings. Am J Respir Crit Care Med *176*(5):498–504.

Zaccone EJ, Thompson JA, Ponnoth DS, Cumpston AM, Goldsmith WT, Jackson MC, Kashon ML, Frazer DG, Hubbs AF, Shimko MJ, Fedan JS [2013]. Popcorn flavoring effects on reactivity of rat airways in vivo and in vitro. J Toxicol Environ Health Part A *76*:669–689.

This page intentionally left blank.

Contents

This page intentionally left blank.

Abbreviations

µg	Microgram
μg^3	Microgram per meter cubed
$\sqrt{Cum(DA)}$	Square root of cumulative exposure
A, B	Subscripts denoting experimental animal and target species, respectively
ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
AH	Absolute humidity
APF	Assigned protection factor
AL	Action level
Arg5	5th arginine
ATS/ERS	American Thoracic Society/European Respiratory Society
Avg(DA)	Average exposure to diacetyl
BFV	Butyric acid in butter flavoring vapors
BLS	Bureau of Labor Statistics
BMC	Benchmark concentration
BMCL	Lower one-sided 95% confidence limit
BMD	Benchmark dose
BMDL	Lower bound on the benchmark dose
BMI	Body mass index
BOOP	Bronchiolitis obliterans organizing pneumonia
BOS	Bronchiolitis obliterans syndrome
Cal/OSHA	California Occupational Safety and Health Administration
CAS	Chemical Abstract Service
CD	Crossdraft
CDC	Centers for Disease Control and Prevention
CDPH	California Department of Public Health
C_{dyn}	Dynamic lung compliance
CFD	Computational fluid dynamic
CFD/PBPK	Computational fluid dynamic/physiologically-based pharmacokinetic model

cfm	Cubic feet per minute
CFR	Code of Federal Regulations
CI	Confidence interval
cm	Centimeter
COP	Cryptogenic organizing pneumonitis
COPD	Chronic obstructive pulmonary disease
CT	Computed tomography
DA	Diacetyl
Cum(DA)	Cumulative exposure to diacetyl
DCXR	Dicarbonyl/L-xylulose reductase
DL_{CO}	Diffusing capacity for carbon monoxide
DNPH	2,4-Dinitrophenylhydrazine
DOT	Department of Transportation
EPA	Environmental Protection Agency
ERS	European Respiratory Society
ET	Extrathoracic, also subscript denoting the extrathoracic region
eV	Electron volt
EX	Exhaust flow rate
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
fpm	Feet per minute
FTIR	Fourier transform infrared gas analyzer
FVC	Forced vital capacity
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
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
g/kg	Grams per kilogram
HCI	Health Canada Initiative
HCS	Health communication standard
HDS	Helium diffusion sampler

HEC	Human equivalent concentration
HEPA	High-efficiency particulate air
HHE	Health hazard evaluation
HPLC-UV	High pressure liquid chromatography- ultraviolet
HRCT	High resolution computed tomography
HSE	Health and Safety Executive
IATA	International Air and Transport Association
ICD	International Classification of Diseases
ICRP	International Commission on Radiological Protection
IOSHA	Indiana Occupational Safety and Health Administration
IR	Infrared
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JEM	Job exposure matrix
K_m	Michaelis constant
LC_{50}	Lethal concentration for 50% of exposed population
LD_{50}	Lethal dose for 50% of dosed population
LEV	Local exhaust ventilation
LLD	Longitudinal limit of decline
L/min	Liters per minute
LLofN	Lower limit of normal
LOD	Limit of detection
LOQ	Limit of quantification
LR	Likelihood ratio
LRT	Likelihood ratio test
mg/mL	Milligrams per milliliter
mg/m^3	Milligrams per meter cubed
mL	Milliliter
mL/min	Milliliters per minute
mM	Millimoles
mV	Millivolt
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NAICS	North American Industry Classification System

ND	Not detected
NHANES III	Third National Health and Nutrition Examination Survey
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute for Environmental Health and Safety
NIOSH	National Institute for Occupational Safety and Health
NTP	National Toxicology Program
OEL	Occupational exposure limit
OR	Odds ratio
OSHA	Occupational Safety and Health Administration
OV-HE	Organic vapor-high efficiency particulate
PAPR	Powered air-purifying respirator
PBPK	Physiologically based pharmacokinetic model
PD	2,3-pentanedione
PEL	Permissible exposure limit
PFT	Pulmonary function test
PTFE	Polytetrafluoroethylene
PID	Photoionization detector
POD	Point of departure
ppb	Parts per billion
PPE	Personal protective equipment
$ppFEV_1$	Percent of predicted FEV_1
ppm	Parts per million
QA	Quality assurance
QC	Quality control
REL	Recommended exposure limit
RfC	Reference concentration
RGDR	Regional gas dose ratio
RH	Relative humidity
R_L	Basal airway resistance
RQL	Reliable quantitation limit
RR	Rate ratio
SA	Surface area (cm^2)
SAR	Supplied-air respirator
SCBA	Self-contained breathing apparatus

SD	Standard deviation
SDS	Safety data sheet
SMR	Standardized mortality ratio
SPIROLA	Spirometry Longitudinal Data Analysis
SPME	Solid-phase microextraction
STEL	Short-term exposure limit
TB	Tracheobronchial region
TD-GC-MS	Thermal desorption-gas chromatography-mass spectrometry
TERA	Toxicology Excellence for Risk Assessment
TLC	Total lung capacity
TWA	Time weighted average
UF	Uncertainty factor
V_E	Minute volume (mL/min = cm^3/min)
VOC	Volatile organic compound

This page intentionally left blank.

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 threshold limit value: 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: Disease processes that show some degree of inflammation, narrowing, or obliteration of small airways (bronchioles) in the lung. Historically, classified into 2 groups: *proliferative* bronchiolitis obliterans and *constrictive* (obliterative) bronchiolitis obliterans which is the type that has been associated with flavorings exposure.

***Constrictive (obliterative) bronchiolitis*:** A potentially fatal, irreversible lung disease with symptoms of dry cough and shortness of breath. On biopsy, the bronchioles are compressed and narrowed by either fibrosis or inflammation. Constrictive (obliterative) bronchiolitis often is characterized by fixed airway obstruction, but pathologic cases sometimes have normal or restrictive spirometry.

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.

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.

***Obliterative bronchiolitis*:** See constrictive bronchiolitis.

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: An 8- or 10-hour time-weighted average, ceiling, or short term exposure concentration recommended by NIOSH that is based on an evaluation of the health effects data.

OSHA permissible exposure limit: Regulatory limits indicating an 8-hour time weighted average exposure unless otherwise noted; a (c) designation denotes a ceiling limit.

Organic vapor cartridge: Devices used in respirators to remove organic vapors from the air.

Priority flavoring *(or priority chemical, substance, etc.):* Generally used in reference to flavoring compounds on the Flavor and Extract Manufacturers Association priority list [FEMA 2004].

Personal protective equipment: Respirators, work gloves, work boots, and other equipment that reduce or eliminate employee 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.

Safety data sheet*:* A listing of a hazardous chemical's health and physical hazards, exposure limits, and precautions (formerly known as material safety data sheet).

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 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% 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.

Short-term exposure limit: Unless otherwise noted, the STEL is the 15-minute TWA exposure that shall not be exceeded at any time during a workday.

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: indicates a time-weighted average concentration for up to a 10-hour work day during a 40-hour workweek.

Volatile organic compound: An organic chemical compound with high vapor pressure and low boiling point.

Acknowledgments

The following document was authored by a multidisciplinary team from six NIOSH divisions. The lead division was the Education and Information Division, Paul Schulte, PhD, Director, Lauralynn Taylor McKernan, ScD, CIH (Manager/Author), and R. Todd Niemeier, MS, CIH (Co-manager/Author).

The following individuals were the authors of the document:

Division of Applied Research and Technology

Kevin H. Dunn, MSEE, CIH
Alberto Garcia, MS
Deborah V.L. Hirst, PhD, PE
Fariba Nourian, BS
Robert P. Streicher, PhD
Jennifer L. Topmiller, MS

Respiratory Health Division

Kathleen B. Fedan, BS
Richard Kanwal, MD (former NIOSH)
Kathleen Kreiss, MD (retired NIOSH)
Greg J. Kullman, PhD, CIH (retired NIOSH)
Chris Piacitelli, MS, CIH (retired NIOSH)

Division of Surveillance, Hazard Evaluations, and Field Studies

Brian Curwin, PhD

Education and Information Division

David A. Dankovic, PhD
Ellen Galloway, MA
Stephen J. Gilbert, MS
Thomas J. Lentz, PhD
Lauralynn Taylor McKernan, ScD, CIH
R. Todd Niemeier, MS, CIH
Robert Park, MS
Randall Smith, MA
Christine Whittaker, PhD

Health Effects Laboratory Division

Jeff Fedan, PhD
Ann F. Hubbs, DVM, PhD, DACVP

National Personal Protective Technology Laboratory

Jay A. Parker, MS, CIH

Special appreciation is expressed to the following individuals for serving as independent, external peer reviewers and providing comments that contributed to the development of this document:

Jonathan Borak, MD, Yale University
Harvey Checkoway, PhD, University of Washington
Mark Cullen, MD, Stanford University
Arthur Frank, MD, PhD, Drexel University
Warren Myers, PhD, West Virginia University
Yiliang Zhu, PhD, University of South Florida
Dale Hattis, PhD, Clark University
Sam Kacew, PhD, ATS, University of Ottawa
David Halton, PhD, CIH, CRSP, Private Consultant
John Howell, PhD, Private Consultant

Additional NIOSH contributors to the document include:

Rachel Bailey, Devin S. Baker, Kenneth Brown, James Couch, Jean Cox-Ganser, Kristin Cummings, Daniel H. Echt, Gino Fazio, Ardith A. Grote, Paul Middendorf, John Piacentino, Stephanie M. Pendergrass, Nikki Romero, Rena Saito, Miriam Siegel, Brian Tift, Doug Trout, Christine Uebel, Abbas Virji, David Weissman, and Vanessa Williams.

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 employee 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 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 a basis for the 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 flavorings-related lung disease through hazard guidance implementation. In their entirety, the RELs and the guidance are intended to help employers develop a more healthful work environment. The RELs 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 employees exposed to diacetyl and other flavoring compounds. 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 the RELs for diacetyl and 2,3-pentanedione. Chapter 8 describes procedures for informing employees about the safety of diacetyl and diacetyl 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 globally harmonized system for classification and labelling (GHS) classifications for diacetyl and 2,3-pentanedione based on the revised OSHA

hazard communication standard. Additionally, recommendations for an effective respiratory protection program are provided. Chapter 9 provides medical surveillance guidelines for the ongoing evaluation of the health status of employees. Chapter 10 describes the components of an effective exposure monitoring program and work practices that when implemented correctly, can reduce occupational exposures. Finally, Chapter 11 presents key research needs.

This document results from a review of all relevant literature on diacetyl and 2,3-pentanedione, and describes selected studies which characterize exposures and discusses techniques shown to be effective in reducing those exposures. Published literature through October 2016 was used and extracted from databases including but not limited to PubMed, NIOSHTIC-2, Web of Knowledge, Toxline, and Chem Abstracts. The literature search was developed to identify critical scientific data relevant to workplace safety and health including physical and chemical properties, human health effects, laboratory testing, chemical toxicokinetics, toxicity, engineering controls, personal protective equipment and function, risk management, and modeling systems that are relevant to diacetyl and 2,3-pentanedione. The literature was searched using specific terminology for each scientific discipline. Evaluated data sources included peer reviewed journal articles, government publications, and peer reviewed data sources, high caliber professional practice manuals (i.e., ACGIH 2012 and FEMA 2012) and high-quality information submitted to government dockets. In a few instances personal communications are cited where authors were contacted for additional clarification. The information that was identified in the comprehensive literature search was evaluated with considerations that included if the studies were peer-reviewed, if the data were generated with standardized protocols, if the exposure conditions were described in detail, confounders and existing information in peer reviewed journals. Specific studies pertaining to workplace exposure assessment, human health effects, and toxicology were specifically identified and are described in Chapters 2, 3, and 4 respectively.

1.3 Background

Diacetyl is one of the main components in butter flavoring that imparts a buttery taste, and it 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. 2008; 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 natural and artificial flavoring ingredient and aroma carrier in bakery products, dairy products, snack foods, and more. It is mainly used as a butter flavoring but is also used in the flavor formulation of a number of other flavors, including but not limited to strawberry, caramel, hazelnut, and butterscotch. It is also present as a natural byproduct in some fermented food products such as beer and roasted food products such as coffee. Occupational exposures in the flavoring and food production industries have been associated with respiratory disease, including obliterative bronchiolitis, an uncommon lung disease often characterized by fixed airways obstruction. Obliterative bronchiolitis 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 compound related to health impairment. Other flavoring ingredients such

as acetaldehyde, butyric acid, and acetoin, have been present in workforces 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.

Day et al. [2011] observed the flavoring compound 2,3-pentanedione in food production facilities. This compound has been used as a diacetyl substitute in many flavor manufacturing facilities because it has a related chemical structure and similar flavor properties to diacetyl. 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. 2012; Morgan et al. 2012].

No state or national registries are available to identify potential cases of obliterative bronchiolitis among employees. In 1985, two employees with fixed obstructive lung disease suggestive of obliterative bronchiolitis were observed in a facility where flavorings with diacetyl were made for the baking industry [Kreiss et al. 2002; NIOSH 1986]. Catastrophic fixed airways disease suggestive of obliterative bronchiolitis was observed in these two former mixing employees who were young nonsmokers with job tasks that involved blending corn starch and flour with various flavorings. Two additional employees 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 compounds.

In the microwave popcorn industry, the first occurrences of obliterative bronchiolitis were observed in the year 2000 when eight employees 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 obliterative bronchiolitis in a separate facility [Parmet 2002]. Since then, numerous cases of obliterative bronchiolitis have been observed in the microwave popcorn industry [Akpinar-Elci et al. 2004; CDC 2002; Ezrailson 2002; Kanwal et al. 2006; NIOSH 2003, 2004a, b, 2006; Parmet 2002; Schachter 2002]. In addition, a retrospective epidemiologic study found cases of obliterative bronchiolitis in employees who were employed in a diacetyl manufacturing plant with exposures to diacetyl, acetoin, acetic acid, and acetaldehyde [van Rooy et al. 2007].

In 2004 and 2006, two cases of obliterative bronchiolitis among employees 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 employees with severe, fixed obstructive lung disease [CDC 2007]. Outreach to the industry regarding the diacetyl hazard, including Cal/OSHA consultation site visits, prompted quick implementation of exposure controls and medical surveillance programs. A longer-term effort was 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 employees at companies using diacetyl [Kim et al. 2010]. In 2010, California issued 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 (vapors, mists, and airborne dusts) and the number of different chemicals used. Although thousands

of flavoring compounds are in use, few have occupational exposure limits. The Flavor and Extract Manufacturers Association (FEMA) reports that of the more than 1,000 flavoring compounds considered to be potential respiratory irritants or hazards, only 46 have established OSHA permissible exposure limits (PELs) [FEMA 2012]. Given the lack of occupational exposure limits for most flavoring compounds, assessing workplace exposures and developing exposure control guidance are critical to help reduce the risk of flavorings-related lung disease.

In 2010, California promulgated a regulation for occupational exposure to food flavorings containing diacetyl that requires installation of exposure controls to reduce exposures to the lowest feasible levels. In 2012, the American Conference of Governmental Industrial Hygienists (ACGIH) published a threshold limit value® of 0.010 ppm 8-hour TWA with a STEL of 0.020 ppm for diacetyl [ACGIH 2012]. In 2014, the European Commission published the Recommendation from the Scientific Committee on Occupational Exposure Limits of 0.02 ppm 8-hour TWA with a STEL of 0.10 ppm for diacetyl [EU 2014].

1.4 Chemical and Physical Properties

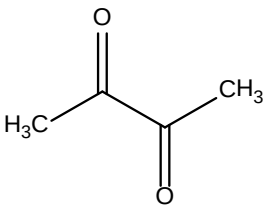
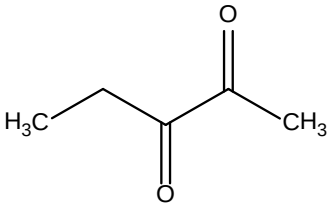
The compound diacetyl has Chemical Abstract Service (CAS) number 431-03-8 and has several synonyms including 2,3-butanedione (International Union of Pure and Applied Chemistry nomenclature), 2,3-butadione, 2,3-diketobutane, biacetyl, dimethyl diketone, and dimethylglyoxal. The compound 2,3-pentanedione has CAS number 600-14-6 and is also referred to by the name acetyl propionyl. Both diacetyl and 2,3-pentanedione are alpha diketones or vicinal diketones (also referred to less specifically as alpha dicarbonyls), which means that their molecular structures contain two carbonyl functional groups that are adjacent to one another, and the carbon molecules attached to the oxygen molecules are also attached to only carbon molecules. A listing of physical and chemical properties of diacetyl and 2,3-pentanedione and their molecular structures is presented in Table 1-1.

The odor threshold of diacetyl and 2,3-pentanedione has been reported by many studies [Buttery et al. 1997; Hall and Andersson 1983; Leksrisompong et al. 2010; Nagata and Takeuchi 1990; Sega et al. 1967]. It is not uncommon for odor threshold values reported in the literature to range over four orders of magnitude for the same chemical [AIHA 1989]. Odor threshold variability can result from the source of data, the characteristics of human olfactory response, and the differences in experimental methodology [AIHA 1989]. The following criteria were used to analyze the diacetyl and 2,3-pentandione odor threshold literature: (1) only primary odor threshold sources that were found in the literature and that were written in English were used; (2) only sources that clearly indicated the type of threshold being measured as a detection or recognition threshold were used; (3) sources that used a panel of at least five judges to account for the range of olfactory sensitivity in the population were used; and (4) sources that presented the different concentrations of odor samples in a way that eliminated olfactory fatigue were used. The geometric mean of the selected values was reported in Table 1-1 [AIHA 1989].

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 into 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;

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 (18°C) [Lide 2008]	0.9565 g/mL (19°C) [Lide 2008]
Refractive index	1.3951 (20°C) [Lide 2008]	1.4014 (19°C) [Lide 2008]
Melting point	−1.2°C [Lide 2008]; −2.4°C [IPCS 2009]; −4°C [Fischer Scientific 2007]	−52°C [Merck Chemicals International 2010]
Boiling point	88°C [Lide 2008]; 88°C [IPCS 2009]	108°C [Lide 2008]; 110°C–112°C [Merck Chemicals International 2010]; 112°C [Chem Service Inc. 1988]
Vapor density	3	3.45
Vapor pressure	52.2 mm Hg (20°C) [Sigma Aldrich 2010]	21.4 mm Hg (20°C) [Merck Chemicals International 2010]
Saturated vapor concentration [Perez 1991]	184 g/m^3 (20°C); 246 g/m^3 (20°C)	117 g/m^3 (20°C)
Water solubility	200 g/L (25°C) [IPCS 2009]	60 g/L (15°C) [Merck Chemicals International 2010]
Flash point, closed cup	6°C [IPCS 2009]; 7°C [Sigma Aldrich 2010]	18°C [Merck Chemicals International 2010]
Autoignition temperature	365°C [IPCS 2009]; 345°C [Sigma Aldrich 2010]	265°C [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]	Fruity/pleasant [Chem Service Inc. 1988]

See footnotes at end of table.

(Continued)

Table 1-1 (Continued). Chemical and physical properties

Property	Diacetyl	2,3-Pentanedione
CAS #	**431-03-8**	**600-14-6**
Odor detection threshold	0.27 ppb [Hall and Andersson 1983; Nagata and Takeuchi 1990] (odor measurement of diacetyl vapor-air mixtures). Note: geometric mean of two literature reported threshold values with a geometric standard deviation of 5.33 ppb. 0.84 ppb [Buttery et al. 1997; Leksrisompong et al. 2010; Sega et al. 1967] (odor measurement of diacetyl vapor in the headspace above aqueous solutions, diacetyl concentrations in solution converted to air concentrations using Henry's Law constant). Note: geometric mean of three literature reported threshold values with a geometric standard deviation of 1.44 ppb. 1.2 ppb [Lawless et al. 1994] (recognition threshold) Note: not a geometric mean because obtained from a single source.	15 ppb [Hall and Andersson 1983] (odor measurement of 2,3-pentanedione vapor-air mixtures). Note: compared to 1.4 ppb for diacetyl obtained from the same reference.¶ 9.4 ppb [Buttery et al. 1997] (odor measurement of 2,3-pentanedione vapor in the headspace above aqueous solutions, 2,3-pentanedione concentration in solution converted to an air concentration using Henry's Law constant). Note: compared to 0.70 ppb for diacetyl obtained from the same reference.¶
Octanol/water partition coefficient, Log P_{ow}	−1.34 [IPCS 2009]	−0.85 [Illovo Sugar Limited 2010]
Henry's Law Constant*	2.95×10^{-5} atm-m^3/mol*; 1.75×10^{-5} atm-m^3/mol at 25°C[Strekowski and George 2005]; 1.35×10^{-5} atm-m^3/mol at 25°C [Betterton 1991]; 1.75×10^{-5} atm-m^3/mol at 25°C [Snider and Dawson 1985]	4.7×10^{-5} atm-m^3/mol*
Appearance	Yellowish green liquid [Merck and Co. Inc. 2006]; green-to-yellow liquid [IPCS 2009]	Dark yellow liquid [Lide 2008]; [yellow to green yellow liquid [OSHA 2014]
Electron impact mass 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]

*Estimated as a ratio of vapor pressure to water solubility

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 a 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 bakery products such as cake mixes, flour and margarines, dairy products such as cheese and yogurt, snack foods such as soft spreads and crackers, beverages such as soft drinks, in addition to candy, 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 solid particulate formulation. Many volatile compounds are also encapsulated in an amorphous carbohydrate, producing more stable products with more manageable properties. Encapsulated powder flavorings are often created with a spray dryer, which converts a slurry mixture into a powder in which the flavorings are surrounded by the powder instead of simply coating the powder. When the encapsulated powder comes into contact with moisture, the flavor is released quickly and completely [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 compounds with similar characteristics, such as 2,3-pentanedione. 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 as a flavor enhancer in the dairy industry. Diacetyl is the major flavor component of starter distillate, constituting as much as 80% to 90% of the mixture's organic flavor compounds [FDA 2009]. A NIOSH health hazard evaluation (HHE) at a modified dairy production company found concentrations of airborne diacetyl ranging up to 2.14 parts per million on a full-shift TWA basis [NIOSH 2009].

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 exposure to diacetyl or diacetyl substitutes 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 2002]. The North American Industry Classification System (NAICS) category 311, the most relevant category, indicates nearly 1.5 million employees are employed in food manufacturing. Bureau of Labor Statistics and Department of Commerce data provide a breakdown of a portion of that number into categories shown in Table 1-2. According to the FEMA, whose members account for approximately 95% of all flavors produced in the United States, a total of 6,520 employees work directly in flavor manufacturing or related laboratory activities in membership companies [Hallagan 2010].

Initial research concerning occupational exposure to diacetyl has focused on employees who directly produce flavorings or use them in the microwave popcorn industry. However, the employment figures for the food production industry suggest that some other employees 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 and Darcey 2002].

Employers in the food manufacturing sector are generally small business owners with 89%

Table 1-2. Breakdown of employees in various categories of the food manufacturing industry

Category description	No. of employees	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]

of establishments employing fewer than 100 employees and nearly 53% of establishments employing fewer than 10 employees [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, 33% of the cheese manufacturing employees employed in the United States were in Wisconsin, and 20% of employees employed in the fruit and vegetable preservation industry were in California [BLS 2007].

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

This page intentionally left blank.

References

29 U.S.C. 669(a)(3). Research and related activities. United States Code. Washington, DC: U.S. Government Printing Office, Office of the Law Revision Counsel.

29 U.S.C. 671(c)(1). National Institute for Occupational Safety and Health. United States Code. Washington, DC: U.S. Government Printing Office, Office of the Law Revision Counsel.

ACGIH [2012]. 2012 TLVs® and BEIs®: threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists Signature Publications.

AIHA [1989]. Odor thresholds for chemicals with established occupational health standards. Dupraz C, Brosseau L, Guy H, May M, Reed L, eds. Fairfax, VA: American Industrial Hygiene Association.

Akpinar-Elci M, Travis WD, Lynch DA, Kreiss K [2004]. Bronchiolitis obliterans syndrome in popcorn production plant workers. Eur Respir J *24*(2):298–302.

Alleman T, Darcey DJ [2002]. Case report: bronchiolitis obliterans organizing pneumonia in a spice process technician. J Occup Environ Med *44*(3):215–216.

Aquila W, Botzem J, Brunner M, Fuchs H, Krause W, Pandl K, Schäfer-Lüderssen U [2001]. Process for the preparation of α-diketones from ketols or ketals from ketols. US Patent 6316676, filed February 28, 2001, and issued November 13, 2001.

Ashley K, McKernan LT, Burroughs E, Deddens J, Pendergrass S, Streicher RP [2008]. Analytical performance criteria. Field evaluation of diacetyl sampling and analytical methods. J Occup Environ Hyg *5*(11):D111–116.

Betterton E [1991]. The partitioning of ketones between the gas and aqueous phases. Atmos Environ *25A*:1473–1477.

BLS (Bureau of Labor Statistics) [2007]. Food manufacturing. http://www.bls.gov/oco/cg/cgs011.htm. Date accessed: October 2007.

BLS (Bureau of Labor Statistics) [2008]. National Employment Matrix, 2008–18, for NAICS 3113, 3115, 3118, and 3119. http://www.bls.gov/emp/ep_table_108.htm. Date accessed: December 2010.

Burdock GA [1997]. Encyclopedia of food and color additives. Boca Raton, FL: CRC Press, p. 2115.

Buttery RG, Ling LC, Stern DJ [1997]. Studies on popcorn aroma and flavor volatiles. J Agr Food Chem *45*(3):837–843.

California Code of Regulations. Title 8, §5197.

CDC (Centers for Disease Control and Prevention) [2002]. Fixed obstructive lung disease in workers at a microwave popcorn factory—Missouri, 2000–2002. MMWR *51*(16):345–347.

CDC (Centers for Disease Control and Prevention) [2007]. Fixed obstructive lung disease among workers in the flavor-manufacturing industry—California, 2004–2007. MMWR *56*(16):389–393.

Chem Service Inc. [1988]. Material safety data sheet, 2,3-pentanedione, O-818.

Day G, LeBouf R, Grote A, Pendergrass S, Cummings K, Kreiss K, Kullman G [2011]. Identification and measurement of diacetyl substitutes in dry bakery mix production. J Occup Environ Hyg *8*(2):93–103.

EPA [2002]. Non-confidential inventory updating report. http://www.epa.gov/iur/tools/data/2002-vol.html. Date accessed: March 2011.

EU [2014]. Recommendation from the Scientific Committee on Occupational Exposure Limits for diacetyl. SCOEL/SUM/149.

Ezrailson EG [2002]. Bronchiolitis in popcorn-factory workers. N Engl J Med *347*(24):1980–1982; author reply 1980–1982; discussion 1980–1982.

FDA (Food and Drug Administration) [2009]. Code of Federal Regulations, Title 21, Volume 3, Part 184, Direct food substances affirmed as generally recognized

as safe, 21 CFR184.1278, diacetyl. U.S. Government Printing Office.

FEMA [2012]. Respiratory health and safety in the flavor manufacturing workplace. In: The flavor and extract manufacturers association of the United States. Washington, DC: p. 28.

Fischer Scientific [2007]. Material safety data sheet, 2,3-Butanedione, 99%. http://fscimage.fishersci.com/msds/03275.htm. Date accessed: November 2009.

Hall G, Andersson J [1983]. Volatile fat oxidation products. I. Determination of odour thresholds and odour intensity functions by dynamic olfactometry. Lebensmittel-Wissenschaft+ Technologie= Food science+ technology.

Hallagan J [2007]. Letter of June 20, 2007, from John Hallagan, General Counsel Flavor Extract Manufacturers Association, to Len Welsh, Acting Director, California Occupational Safety and Health Administration, distributed at the California OSHA Public Advisory Meeting, July 10, 2007.

Hallagan J [2010]. Letter of October 19, 2010, from John Hallagan, General Counsel Flavor Extract Manufacturers Association, based upon 2010 Industry Survey, to L. McKernan, Education and Information Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Department of Health and Human Services.

Hubbs AF, Cumpston AM, Goldsmith WT, Battelli LA, Kashon ML, Jackson MC, Frazer DG, Fedan JS, Goravanahally MP, Castranova V, Kreiss K, Willard PA, Friend S, Schwegler-Berry D, Fluharty KL, Sriram K [2012]. Respiratory and olfactory cytotoxicity of inhaled 2,3-pentanedione in Sprague-Dawley rats. Am J Pathol *181*(3):829–844.

Illovo Sugar Limited [2010]. Pentanedione, safety data sheet according to EC regulation No. 1907/2006.

IPCS (International Programme on Chemical Safety) [2009]. International Chemical Safety Card, 2,3-Butanedione. http://www.inchem.org/documents/icsc/icsc/eics1168.htm. Date accessed: March 2014.

Kanwal R [2003]. Letter of July 2, 2003, from R. Kanwal, Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Department of Health and Human Services, to Frank Morrison, Nebraska Popcorn. Clearwater, NE.

Kanwal R, Kullman G, Piacitelli C, Boylstein R, Sahakian N, Martin S, Fedan K, Kreiss K [2006]. Evaluation of flavorings-related lung disease risk at six microwave popcorn plants. J Occup Environ Med *48*(2):149–157.

Kanwal R, Martin S [2003]. Letter of May 13, 2003, from R. Kanwal and S. Martin, Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Department of Health and Human Services, to Keith Heuermann, B.K. Heuermann Popcorn, Inc. Phillips, NE.

Kim T, Materna B, Prudhomme J, Fedan K, Enright PL, Sahakian N, Windham G, Kreiss K [2010]. Industry-wide medical surveillance of California flavoring manufacturing workers: cross-sectional results. Am J Ind Med *53*(9):857–865.

Kreiss K, Gomaa A, Kullman G, Fedan K, Simoes EJ, Enright PL [2002]. Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. N Engl J Med *347*(5):330–338.

Lambrecht S, Franke O, Zahlman K [2004]. Process for the preparation of 2,3-pentanedione. U.S. Patent 6720456, filed November 7, 2002, and issued April 13, 2004.

Lawless HT, Antinone MJ, Ledford RA, Johnston M [1994]. Olfactory responsiveness to diacetyl. J Sens Stud *9*(1):47–56.

Leksrisompong P, Barbano DM, Foegeding AE, Gerard P, Drake M [2010]. The roles of fat and pH on the detection thresholds and partition coefficients of three compounds: diacetyl, δ-decalactone and furaneol. J Sens Stud *25*(3):347–370.

Lide D, ed. [2008]. CRC handbook of chemistry and physics. Boca Raton, FL: CRC Press.

Lockey JE, Levin LS, Lemasters GK, McKay RT, Rice CH, Hansen KR, Papes DM, Simpson S, Medvedovic M [1998]. Longitudinal estimates of pulmonary function in refractory ceramic fiber manufacturing workers. Am J Respir Crit Care Med *157*(4 Pt 1):1226–1233.

Martyny JW, Van Dyke MV, Arbuckle S, Towle M, Rose CS [2008]. Diacetyl exposures in the flavor manufacturing industry. J Occup Environ Hyg *5*(11):679–688.

Merck and Co. Inc., ed. [2006]. The Merck index. 14th ed. Whitehouse Station, NJ: Merck and Co. Inc.

Merck Chemicals International [2010]. 2,3-Pentanedione. http://www.merck-chemicals.com/2-3-pentanedione/MDA_CHEM-818555/p_HMKb.s1LnGcAAAEWhuEfVhTl]. Date accessed: December 2010.

Miller DJ, Perry SM, Fanson PT, Jackson JE [1998]. Condensed phase preparation of 2,3-pentanedione. U.S. Patent 5831130, filed April 24, 1997, and issued November 3, 1998.

Morgan DL, Jokinen MP, Price HC, Gwinn WM, Palmer SM, Flake GP [2012]. Bronchial and bronchiolar fibrosis in rats exposed to 2,3-pentanedione vapors: implications for bronchiolitis obliterans in humans. Toxicol Pathol *40*(3):448–465.

Nagata Y, Takeuchi N [1990]. Measurement of odor threshold by triangle odor bag method. Bull Jpn Environ Sanit Cent *17*:77–89.

National Toxicology Program [1994]. 2,3-Butanedione. CAS Number 431-03-8. http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/431-03-8.pdf. Date accessed: December 2010.

National Toxicology Program [2007]. Chemical information review document for artificial butter flavoring and constituents: diacetyl [CAS No. 431-03-8] and acetoin [CAS No. 513-86-0]. http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/Artificial_butter_flavoring.pdf. Date accessed: March 2011.

NIOSH [1986]. Hazard evaluation and technical assistance report: International Bakers Services, Inc, South Bend, IN. By McConnell R, Hartle R. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 1985–0171–1710.

NIOSH [2003]. Hazard evaluation and technical assistance report: Agrilink Foods Popcorn Plant, Ridgway, IL. By Sahakian N, Choe K, Boylstein R, Schleiff P. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2002–0408–2915.

NIOSH [2004a]. Hazard evaluation and technical assistance report: American Pop Corn Company, Sioux City, IA. By Kanwal R, Boylstein R, Piacitelli C. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2001–0474–2943.

NIOSH [2004b]. Hazard evaluation and technical assistance report: ConAgra Snack Foods, Marion, OH. By Kanwal R, Kullman G. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2003–0112–2949.

NIOSH [2006]. Hazard evaluation and technical assistance report: Gilster-Mary Lee Corporation, Jasper, MO. By Kanwal R, Kullman G, Fedan K, Kreiss K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2000–0401–2991.

NIOSH [2009]. Hazard evaluation and technical assistance report: Chr. Hansen, Inc., New Berlin, WI. By Sahakian N, Kullman G, Dunn K, Kanwal R. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2007–0327–3083.

Nottingham University [1983]. Eight peak index for mass spectra. 3rd ed. Nottingham, UK: The Mass Spectrometry Data Centre, The Royal Society of Chemistry.

OSHA [2014]. Chemical sampling information, 2,3 pentanedione. https://www.osha.gov/dts/chemicalsampling/data/CH_260240.html. Date accessed: September 2014.

Parmet AJ [2002]. Bronchiolitis in popcorn-factory workers. N Engl J Med *347*(24):1980–1982; author reply 1980–1982; discussion 1980–1982.

Parmet AJ, Von Essen S [2002]. Rapidly progressive, fixed airway obstructive disease in popcorn workers: a new occupational pulmonary illness? J Occup Environ Med *44*(3):216–218.

Pouchert CJ, ed. [1985]. The Aldrich library of FT-IR spectra. Edition I, Volume I. Milwaukee, WI: Aldrich Chemical Co.

Saraiva MA, Borges CM, Florencio MH [2006]. Non-enzymatic model glycation reactions: a comprehensive study of the reactivity of a modified arginine with aldehydic and diketonic dicarbonyl compounds by electrospray mass spectrometry. J Mass Spectrom *41*(6):755–770.

Schachter EN [2002]. Popcorn worker's lung. N Engl J Med *347*(5):360–361.

Sega GM, Lewis MJ, Woskow MH [1967]. Evaluation of beer flavor compounds. Proceedings of the annual meeting of the ASBC *25*:156–164.

Sigma Aldrich [2010]. Material safety data sheet, No. B85037, 2,3- butanedione. http://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=D3634&brand=SIGMA&PageToGoToURL=http%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigma%2Fd3634%3Flang%3Den. Date accessed: December 2009.

Snider JR, Dawson GA [1985]. Tropospheric light alcohols, carbonyls, and acetonitrile: concentrations in Southwestern United States and Henry Law data. J Geophysical Res: Atmos *90*(ND2):3797–3805.

Strekowski RS, George C [2005]. Measurement of Henry's Law Constants for acetone, 2-butanone, 2,3-butanedione, and isobutyraldehyde using a horizontal flow reactor. J Chem Eng Data *50*(3):804–810.

Ubbink J, Schoonman A [2002]. Flavor delivery systems. In: Kirk RE, Othmer DF, eds. Kirk-Othmer encyclopedia of chemical technology. 4th ed. Cary, NC: John Wiley and Sons, Inc., pp. 527–563.

United States Census Bureau, United States Department of Commerce [2004]. Spice and Extract Manufacturing. 2002 Economic Census. Manufacturing Industry Series. Volume: EC02–311942(RV).

van Rooy FG, Rooyackers JM, Prokop M, Houba R, Smit LA, Heederik DJ [2007]. Bronchiolitis obliterans syndrome in chemical workers producing diacetyl for food flavorings. Am J Respir Crit Care Med *176*(5):498–504.

2 Assessing Occupational Exposure in Employees

2.1 Introduction

Measurement of diacetyl and 2,3-pentanedione exposure is helpful in preventing flavorings-related lung disease, even with complex flavorings formulations. Exposures to diacetyl and 2,3-pentanedione can be monitored using personal and area (environmental) air samples because the predominant route of exposure is inhalation. Results from air sampling can be compared with established criteria such as the NIOSH RELs. Measuring employees' exposures to diacetyl or 2,3-pentanedione may help identify processes, locations, or tasks with exposures of concern; guide corrective actions such as engineering controls; identify improved work practices; and select appropriate respiratory protection.

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 dust and bulk materials; (3) real-time techniques for measuring relevant airborne analytes and other flavoring compounds; and (4) 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 Appendices A–E.

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 employee exposure. For personal sampling, an employee wears the air sampling equipment, and the inlet to the collection medium is positioned within the employee's breathing zone. Area sampling is performed for several purposes such as to evaluate exposure characteristics associated with an area or process, and 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 employee throughout the sampling period.

2.2.1 OSHA Methods 1012 and 1013

In response to the need for longer sampling time periods with a lower limit of detection or reliable quantitation limit, the Occupational Safety and Health Administration (OSHA) validated two sampling and analytical methods, OSHA Method 1012 and OSHA Method 1013, for diacetyl and acetoin in 2008 [OSHA

2008a, b]. OSHA Method 1013 is for monitoring low ppm levels, while OSHA Method 1012 is for monitoring ppb levels [OSHA 2008b]. These methods can be used for the simultaneous determination of diacetyl and acetoin. As of the publication of this document, these are the recommended methods for diacetyl.

OSHA Methods 1012 and 1013 use two 600 milligram (mg) sorbent tubes containing specially cleaned and dried silica gel (SKC Inc., Eighty Four, PA, Catalog no. 226-183) in series and air is sampled at a flow rate of 50 milliliters per minute (mL/min) for up to 180 minutes for determination of TWA concentrations, and a flow rate of 200 mL/min for 15 minutes for short-term concentration measurements. An opaque sampling tube protective cover should be used in conjunction with the sampler to prevent the glass sampling tube from breaking and to protect the sample from light, which can decompose diacetyl and acetoin. After sampling, the tubes should be separated, capped, and protected from light with aluminum foil or other opaque material. There is no requirement that samples be kept cold during shipping or storage.

OSHA Method 1013 has a reliable quantitation limit of 12 ppb (0.041 mg/m^3) diacetyl for a 9-liter sample, and samples are analyzed by gas chromatography using a flame ionization detector (GC-FID). OSHA Method 1012 has a nearly 10 times lower RQL of 1.3 ppb (4.57 micrograms per meter cubed [µg/m^3]) diacetyl for a 9-liter sample, which is achieved by derivatizing diacetyl with 2 milligram per milliliter (mg/mL) O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride in the extraction solution and analyzing by gas chromatography using an electron capture detector (GC-ECD). An advantage of OSHA Method 1013 is that sample preparation can be performed in one hour, whereas the derivatization step of OSHA Method 1012 requires 36 hours. After samples have been extracted and analyzed using OSHA Method 1013, if needed (e.g., if sample concentration is not detectable), they can be derivatized and analyzed using OSHA Method 1012 to benefit from its lower detection capability.

2.2.2 OSHA Method 1016

OSHA Method 1016 [OSHA 2010] can be used to measure 2,3-pentanedione concentrations. OSHA Method 1016 uses the same sampling media, sample collection procedure and analytical procedure as OSHA method 1013. However, OSHA Method 1016 allows for the simultaneous analysis of 2,3-pentanedione, diacetyl, and acetoin by using a different analytical column to optimize the analytical separation of these compounds. In addition, OSHA Method 1016 requires samples to be shipped cold. If diacetyl and/or acetoin are not anticipated to be present, OSHA Method 1016 can be used to sample for an additional 20 minutes, or 200 minutes, at 50 mL/min to determine TWA concentrations of 2,3-pentanedione [OSHA 2010]. For a 10-liter sample, the RQL of 2,3-pentanedione is 9.3 ppb (38 µg/m^3).

2.2.3 OSHA Method PV2118

Superseded by OSHA Methods 1012 and 1013, OSHA Method PV2118 [OSHA 2003] was developed as an air sampling method for diacetyl that uses two 150/75 mg silica gel sorbent tubes in series (SKC Cat. No. 226-10) at a recommended flow rate of 50 mL/min for one hour. In response to the limited capacity of this sampler in humid environments, a modified version of OSHA Method PV2118 was used by some practitioners in the field. The modified method uses larger 400/200 mg sorbent tubes packed with specially cleaned silica gel (SKC Cat. No. 226-10-03) allowing for greater

sample capacity without breakthrough of diacetyl. Sample analysis remained unchanged.

2.2.4 NIOSH Method 2557

While no longer recommended for use, NIOSH developed NIOSH Method 2557 [NIOSH 1994] for measuring diacetyl vapor in air. It called for the collection of samples onto a 150/75 mg carbon molecular sieve sorbent tube (Cat. No. 226-121, SKC Inc., Eighty Four, PA) at a flow rate between 10 and 200 mL/min for a sample volume between 1 and 10 liters. The method specifies that samples be stored cold and analyzed within 7 days of sampling.

Until 2007, NIOSH Method 2557 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. 2008]. In 2007, field and chamber investigations indicated that NIOSH Method 2557 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. 2008]. Side-by-side field samples were collected in flavor manufacturing facilities and analyzed according to NIOSH Method 2557, OSHA Method PV2118, and a modified version of OSHA Method PV2118. The results of this field work confirmed the tendency of NIOSH Method 2557 to underestimate the true concentration of diacetyl as humidity increases. 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). Controlled test atmospheres of diacetyl were generated and sampled through an array of sampling tubes at calibrated flow rates. Test atmospheres were controlled for diacetyl concentration, temperature, and relative humidity. Results indicated that diacetyl recoveries for NIOSH Method 2557 were affected by absolute humidity (AH), storage time of sample tube prior to extraction, and diacetyl air concentration. The study resulted in the development of a mathematical procedure to adjust diacetyl concentrations previously measured using NIOSH Method 2557. The procedure is presented in Appendix F 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 occupational exposure to flavoring compounds, new methods continue to be developed for their measurement. At this time, however, none of these methods are validated.

A method is being developed by NIOSH to measure alpha-dicarbonyl compounds (such as diacetyl and 2,3-pentanedione) in air via derivatization with 1,2-phenylenediamine. This compound 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. Samples are extracted in the lab and extraction solutions 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 that would be experienced if sampling for an extended time with uncoated silica gel tubes. Experiments to date indicate

no breakthrough of diacetyl, 2,3-pentanedione, or 2,3-hexanedione from the sampling tubes after passing 144 liters of air at 80% RH. This enables sampling for 8 hours without changing out sampling tubes. Another advantage is the high sensitivity of NPD detection, which will enable measurement of alpha-dicarbonyl compounds below the proposed REL for diacetyl of 5 ppb.

A new method for collecting air samples using evacuated canisters has been evaluated for several VOCs [LeBouf et al. 2012]. The 450-milliliter canisters, which can be equipped with either instantaneous grab sampling attachments or restricted-flow controllers (for task-based or full-shift sampling), are suitable for collection of area and personal samples. The air samples are analyzed for VOCs using a preconcentrator/gas chromatography-mass spectrometry (GC-MS) system. At present, this canister method is in the process of being validated with three additional compounds, diacetyl, 2,3-pentanedione and 2,3-hexanedione, and is being reviewed for incorporation into the *NIOSH Manual of Analytical Methods*.

A method for priority flavoring compounds is being investigated that utilizes a novel sampler—the helium diffusion sampler (HDS) [Entech Instruments Incorporated 2011]. The HDS collects a whole air sample for either short-term or full-shift sampling. The advantages of HDS are that no air sampling pump is required, 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 a preconcentrator/GC-MS in the selected ion monitoring mode. Although HDS will not support limits of detection achieved by TD-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 sorbent tubes can provide a high degree of sensitivity. This is because desorption of compounds from thermal desorption tubes does not involve dilution into an extraction solvent. Instead, compounds are thermally desorbed from the sampling tubes in a thermal desorption system. This technique is primarily used for qualitative screening purposes because of the ability of thermal desorption tubes to capture a diverse range of VOCs, but specific compounds can be quantified if corresponding standards are analyzed along with the samples. The thermal desorption tube is usually a stainless steel tube configured and filled with a single sorbent bed or multiple beds of various sorbents including carbonaceous materials, carbon molecular sieves, and/or porous polymers. The sorbents can be heated to high temperatures without breakdown or the generation of artifacts, so thermal desorption tubes can be cleaned and reused multiple times. The tubes are analyzed with a thermal desorber-GC-MS (TD-GC-MS) [NIOSH 1994].

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

Although diacetyl and 2,3-pentanedione are normally found in liquid form, they can also be encapsulated in or coated on a powder substrate. Air sampling for dust that may be generated during handling of powdered flavorings can be achieved by active sampling methods. During the sample collection, however, some of the diacetyl and 2,3-pentanedione may volatilize,

i.e., release from the dust particles and enter the vapor phase due to contact with moisture. In addition, environments in which dust is generated may also contain vapors of the flavoring compounds. Sorbent tubes used for the collection of vapor-phase diacetyl or 2,3-pentanedione cannot be used to adequately sample for dust at the low flow rates required by the tubes [OSHA 2008a]. As a result, modifications to the sampling methods are necessary to assess exposure to both vapor and dust.

2.3.1 Size-Selective Air Sampling for Dust

Measurement of airborne dust particles according to their size (e.g. inhalable, thoracic, and respirable) can help to understand where they may deposit in the respiratory tract. Several types of sampling devices are available (e.g., inhalable dust samplers, impactors, cyclones, and sampling cassettes) to provide measurements of different size fractions of airborne dust. In most cases, dust is collected onto a filter, and the filter can be analyzed via gravimetric means to provide the mass of the dust. Filters should be hydrophobic in nature (e.g., polyvinyl chloride) in order to minimize collection of moisture. After being measured gravimetrically, filters can be analyzed for diacetyl and other compounds by the procedure described in section 2.3.2. Validated methods such as NIOSH Method 0500 for total dust and NIOSH 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 the quantitative measurement of diacetyl, 2,3-pentanedione, and potentially other flavoring compounds in dust. A sampling cassette with a filter is used to collect airborne dust. The filter is then extracted in water and the aqueous solution is heated to promote the transfer of volatile components to the headspace above the solution. The headspace is sampled using a solid-phase microextraction (SPME) fiber. Headspace SPME involves an equilibrium process in which the volatile analytes establish equilibria between the sample solution, the headspace above the solution, and the polymer-coated fused silica fiber. The mechanism by which the analytes are extracted from the headspace is based on absorption of the analytes onto the fiber. The fiber is inserted directly into a GC-MS. The analytes are extraced from the fiber in the hot injection port and concentrated onto an analytical column. Because the entire sample collected on the fiber is introduced into the GC-MS instrument, as opposed to an aliquot of the sample for methods in which a solvent extract is used, lower detection limits can be achieved. This same procedure can be used to measure diacetyl, 2,3-pentanedione, and potentially other flavoring compounds in samples of bulk powders.

2.3.3 Bulk Liquids and Solids

2.3.3.1 Sample collection

Although the review of safety data sheets or other available product documentation may be helpful to identify flavor compounds and potential exposures, they are not always comprehensive or specific. Collection and analysis of bulk flavoring materials can be useful to identify and quantify chemical ingredients and guide exposure assessment strategies. Prior to collecting bulk samples, it is important to consider the physical state of the materials to be sampled (liquids, pastes, or powders), the need to sample opened or unopened containers, the sampling locations, the number of samples to collect, and the amount of sample to collect (often determined by requirements of the laboratory analysis). Bulk samples should

be representative; in other words, they should be derived from a variety of sampling locations and obtained from multiple batches to capture any variability in the bulk materials used.

When sampling, it is important to collect and transport the sample in a manner that does not contaminate or cross-contaminate the bulk materials. Only clean or unused sample containers that are compatible with the bulk materials sampled should be used. In general, glass containers are ideal because they will not react with most chemicals, but polyethylene or polypropylene containers may also be appropriate. A typical container is a 20-mL glass scintillation vial with a polytetrafluoroethylene (PTFE)-lined screw cap. Each container should be clearly labeled with information about the bulk sample including material sampled, company and product number, site of sampling, date of sampling, sample tracking number and any hazards or precautions to be taken when handling the bulk sample.

After sampling, consideration should be given to preserve the integrity of the bulk samples during storage and shipping. For example, care should be taken to keep samples cold and protected from light if necessary. In addition, bulk materials should not be shipped together with air samples. Established Department of Transportation (DOT) and International Air and Transport Association (IATA) shipping regulations of hazardous materials and dangerous goods should be followed if hazardous materials are to be shipped. Materials that are considered hazardous for the purpose of transportation under the DOT regulations are listed in the hazardous materials table in Title 49 of the Code of Federal Regulations (CFR), Section 172.101 [49 CFR 172.101]; materials that are considered dangerous goods for the purpose of shipping by air under IATA regulations are listed in the list of dangerous goods in IATA dangerous goods regulations, section 4.2 [IATA 2012]. The DOT and IATA regulations guide the classification/identification and packaging of hazardous materials and the marking and labeling of shipping containers containing hazardous materials. If the materials to be shipped are known to be hazardous but the specific names of the materials are not found on either the DOT hazardous materials table or the IATA list of dangerous goods, then the materials must be classified into a hazard class according to section 3 of the IATA dangerous goods regulations handbook, and a proper shipping name must be assigned according to section 4 of the IATA dangerous goods regulations handbook. A person must be trained in DOT and IATA regulations and certified in order to mark a shipment as hazardous. If it is unknown whether the materials to be shipped are hazardous or not, then a person who is trained in DOT and IATA regulations should be consulted.

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 Compounds

Several analytical methods provide real-time or near real-time measurements of volatile compounds in 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) can be used to monitor VOC air concentrations in 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 electron volts (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. Because 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 2,000 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 preloaded factory calibrations for over 100 gases, but because 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. Many MIRAN 1A models are 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 gas analyzer (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 compounds in air providing chemical specific full-shift, partial-shift, and peak concentration measures although interferences can pose analytical difficulties in quantifying specific flavoring compounds 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 measured 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 that 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 2-1.

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.

In 2010, a microwave popcorn company asked NIOSH to evaluate chemical constituents in eight liquid butter flavorings because their supplier did not identify chemical substitutes they were using in place of diacetyl [Boylstein 2012]. Quantitative GC-MS analysis showed acetoin in five samples, 2,3-pentanedione in four, and 2,3-hexanedione in one, all at concentrations of 0.5% or less by weight, except for one acetoin sample at 2%. The more sensitive semiquantitative headspace analysis with thermal detection tubes found diacetyl and acetoin in all samples, 2,3-pentanedione in five, 2,3-hexanedione in one, and 2,3-heptanedione in one.

2.5.2 Other Microwave Popcorn Production Exposure Assessments

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 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 1986]. Case histories showed severe fixed

Table 2-1. Multiple investigations of diacetyl in flavoring and food production industries

Study	Method*	Location	Diacetyl concentration in ppm (sample type)		
			Arithmetic mean	Geometric mean	Range
Microwave popcorn plants					
Facility G (first survey) [NIOSH 2006]	NIOSH Method 2557 (corrected)	Mixing room	57.2 (full-shift TWA)	_	_
		Packaging area	2.8 (full-shift TWA)	_	_
		QC lab	0.8 (full-shift TWA)	_	_
		Maintenance	0.9 (full-shift TWA)	_	_
		Other areas	< 0.15 (full-shift TWA)	_	_
Facilities G,J,K,L,N,O [Kanwal et al. 2006]	NIOSH Method 2557 (corrected)	Packaging areas (area samples)	0.019–3.0	_	_
		Packaging areas (personal samples)	0.023–1.16	_	_
		Mixing rooms/areas (area samples)	0.63–57.2	_	_
		Mixing rooms/areas (personal samples)	0.035–1.33	_	_
[White 2011]	NIOSH Method 2557 (corrected)	Mixers	0.119–2.704 (full shift)	0.044–0.587 (full shift)	0.004–11.72 (full shift)
		Non-mixers	0.042–0.123 (full shift)	< 0.001–0.019 (full shift)	0.004–1.984 (full shift)
Flavoring production plants					
Facility B [NIOSH 2007a]	NIOSH Method 2557 (corrected)	Powdered flavoring production area	2.73 (full-shift TWA)	_	204 (real-time peak)
			25.9 (partial shift)	_	_
Facility C [NIOSH 2008b]	OSHA Method PV2118	Liquid flavoring production area	0.46 (full-shift TWA)	_	_
		Powdered flavoring production area	0.34 (full-shift TWA)	_	_
		Task-based (pouring diacetyl)	_	_	11 (10-minute peak)
Facility H [NIOSH 2008a]	OSHA Method PV2118	Liquid production room (area samples)	0.26 (full-shift TWA)	_	_
		Powder production room (area samples)	0.07 (full-shift TWA)	_	_
	NIOSH Method 2557	Liquid production (personal samples)	0.10	_	_
		Powder production (personal samples)	0.05	_	_

See footnotes at end of table.

(Continued)

Table 2-1 (Continued). Multiple investigations of diacetyl in flavoring and food production industries

Study	Method*	Location	Diacetyl concentration in ppm (sample type)		
			Arithmetic mean	Geometric mean	Range
Facility D [NIOSH 2009c]	OSHA Method PV2118	Starter distillate room	_	1.06 (full-shift TWA)	1.06 (full-shift TWA)
		Starter distillate room (personal samples)	_	1.78 (full-shift TWA)	1.78 (full-shift TWA)
		Spray dry room	_	1.07 (full-shift TWA)	1.07 (full-shift TWA)
		Spray dry room (personal samples)	_	0.756 (full-shift TWA)	0.756 (full-shift TWA)
		Flavors room	_	0.171 (full-shift TWA)	0.171 (full-shift TWA)
		Flavors room (personal samples)	_	0.329 (full-shift TWA)	0.329 (full-shift TWA)
		Spray dry room, task-based (moving diacetyl between containers)	_	_	_
		Spray dry room, task-based (cleaning barrel with hose)	_	_	_
Facility I [NIOSH 2011]	NIOSH Method 2557	Spray drying	_	0.169 (full-shift TWA)	_
		Spray drying (personal samples)	_	0.123 (full-shift TWA)	_
		Other production areas	_	0.375 (full-shift TWA)	_
		Other production areas (personal samples)	_	0.762 (full-shift TWA)	_
	OSHA Method PV2118	Spray drying	_	0.167 (full-shift TWA)	_
		Spray drying (personal samples)	_	0.182 (full-shift TWA)	_
		Coffee and tea area	_	0.076 (full-shift TWA)	_
		Liquid compounding area (personal samples)	_	1.900 (full-shift TWA)	_
Facility Q [NIOSH 2013a]	Canister	Multiple locations (area samples)	_	_	No diacetyl detected (< 0.0029)(instant to 3 hrs)
[Martyny et al. 2008]	NIOSH Method 2557	All areas (personal samples)	2.48 (1–3 hours)	_	0.01–60 (1–3 hours)
Diacetyl production					
[van Rooy 2007]		Task specific	_	_	0.6–83 (real-time peaks)

See footnotes at end of table.

(Continued)

Table 2-1 (Continued). Multiple investigations of diacetyl in flavoring and food production industries

Study	Method*	Location	Diacetyl concentration in ppm (sample type)		
			Arithmetic mean	Geometric mean	Range
Food production					
Facility M -popped corn [NIOSH 2007b]	NIOSH Method 2557	All areas Directly above heated popping oil	– –	– –	No diacetyl detected(< 0.01) 0.14 (real-time peak)
Facility E -bakery mix [NIOSH 2009a]	OSHA Methods PV2118, 1013	All areas	–	–	No diacetyl detected
Facilities F (3 sites)- cafeterias [NIOSH 2009b]	OSHA Method PV211	All areas	–	–	No diacetyl detected (LOD< 0.02)
Facility P -cream cheese [NIOSH 2013b]	OSHA Method 1012	Cooking, filling, packaging (personal samples) Cooking, filling, packaging (area samples)	– –	– –	0.0004–0.0083 (full shift) 0.0003–0.0138 (full shift)
Facility U -snack food [NIOSH 2013c]	Canister	Snack chip production (personal samples) Snack chip production (area samples)	– –	– –	No diacetyl detected (LOD varied from < 0.0028– < 0.006) (2–3 hrs) 0.0014–0.0017 (instant)
Facility R-coffee [Bailey et al. 2015; Duling et al. 2016]	OSHA Method 1012	Warehouse (personal samples)	0.008 (full-shift TWA)	_	_
		Roasting room (personal samples)	0.026 (full-shift TWA)	_	_
		Grinding/packaging room (personal samples)	0.093 (full-shift TWA)	_	_
				_	_
		Flavoring room (personal samples)	0.080 (full-shift TWA)	_	_
		Other areas (personal samples)	0.007–0.081(full-shift TWA)	_	_
		Warehouse (area samples)		_	_
		Roasting room (area samples)	0.011 (full-shift TWA)	_	_
		Grinding/packaging room (area samples)	0.020 (full-shift TWA)	_	_
		Flavoring room (area samples)	0.104 (full-shift TWA)	_	_
		Other areas (area samples)	0.090 (full-shift TWA)	–	_
		Packaging room (area sample)	0.004–0.062(full-shift TWA)	–	14.3 (15-minute)

*(corrected) indicates concentration values have been corrected for humidity and time to extraction effects
_ no data collected

obstructive lung disease among employees in a mixing room. Data from previous air monitoring indicated a high dust concentration in the personal breathing zone of an employee 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 employees' disease was most likely caused by some agent in the mixing room at the plant.

NIOSH personnel 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. Employees' 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 an employee 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 PID) and dust (as measured with personal dust monitors) with time.

A health hazard evaluation was conducted at a facility in Wisconsin [NIOSH 2009c] 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 employee'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 an employee 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 2011]. 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).

Another health hazard evaluation was performed at a flavorings plant in Kentucky that produced flavors, colors, and food and beverage ingredients used in the manufacture of consumer products [NIOSH 2013a]. Diacetyl was not found in use during the NIOSH air sampling survey. Using evacuated canisters, diacetyl and 2,3-hexanedione were not detected in any of the instantaneous or 3-hour area air samples taken in several parts of the plant. 2,3-Pentanedione was detected in two area air samples taken in the liquid samples room. The detection limits ranged from 1.4 to 2.9 ppb for diacetyl, 1.5 to 3.2 ppb for 2,3-pentanedione, and 1.7 to 3.6 ppb for 2,3-hexanedione. Of the two air samples that detected 2,3-pentanedione in the room, one was an instantaneous sample taken near a trash can for disposal of used pipettes while making a flavoring recipe and resulted in a level of 47 ppb. The other sample that detected 26 ppb 2,3-pentanedione was collected for 187 minutes in the center of the room. During the sampling period, several employees were preparing recipes, which included fruit and cheese flavors.

2.5.4 Other Flavoring Manufacturing Exposure Assessments

In a study evaluating diacetyl exposures in 16 flavor manufacturing facilities, 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 unsampled 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 2009a], a popcorn popping plant [NIOSH 2007b], three office building cafeterias [NIOSH 2009b], a cream cheese manufacturing plant [NIOSH 2013b], a snack food production plant [NIOSH 2013c], and a coffee production plant [Bailey et al. 2015; Duling et al. 2016].

At the bakery mix production facility, employees 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 plant 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, in-tube derivatization with 1,2-phenylenediamine (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.

At the cream cheese plant in 2011, several flavorings, including dairy, cheese, strawberry, blueberry, and smoke were found with headspace sampling to contain diacetyl with or without 2,3-pentanedione. Air sampling with OSHA Method 1012 during cooking, filling, and packaging of cream cheese made with some of those flavorings, measured area diacetyl concentrations (n=15 near full-shift) from

0.3 to 13.8 ppb. The four highest concentrations were greater than 11 ppb: three of these were collected in a cooking area and the other in a filling area. Fourteen near full-shift personal diacetyl exposures ranged from 0.4 to 8.3 ppb, while six short-term samples collected mostly while ingredients were added to cook kettles ranged from 4.4 to 15.1 ppb. Fourteen area and six personal concentrations of 2,3-pentanedione measured with OSHA Method 1016 were all less than limits of detection (15.8 to 48.8 ppb), as were two of the three also sampled with the more sensitive draft NIOSH method using 1,2-phenylenediame-treated silica gel tubes (0.5 ppb limit of detection) – the detectable concentration was 0.9 ppb while using smoke flavoring. Of six area samples collected alongside cleaning operations with evacuated canisters for 2,3-pentanedione (1.2 to 2.9 ppb limits of detection) and 2,3-hexanedione (1.5 to 3.6 ppb limits of detection), one measured 2,3-pentanedione at 6.2 ppb and 2,3-hexanedione at 9.0 ppb during a nearly 3-hour cleaning procedure of cooking equipment containing strawberry cream cheese remnants while no cream cheese was being made in the room.

The snack food production plant applied powdered seasonings onto potato, corn, and tortilla chips after they were fried. Headspace analyses of bulk samples of seasonings found trace amounts of diacetyl, but no other alpha-diketone compounds, in four of the seven samples: barbeque, honey barbeque, cheddar sour cream, and chili cheese. Diacetyl, 2,3-pentanedione, and 2,3-hexanedione were not detected in the five 15- to 180-minute personal breathing zone evacuated canister air samples from processing line operators during nacho cheese tortilla chip production. The detection limits ranged from 2.8 to 6.0 ppb for diacetyl, 3.4 to 7.2 ppb for 2,3-pentanedione, and 3.2 to 6.8 ppb for 2,3-hexanedione. Although diacetyl was detected in three area samples collected instantaneously near the seasoning hopper, it was not quantifiable. Because it was found between the detectable level of 1.3 ppb and the quantifiable level of 4.3 ppb, the reported concentrations of 1.4 to 1.7 ppb are considered estimates. The area samples did not detect 2,3-pentanedione or 2,3-hexanedione (detection limits of 1.5 and 1.6 ppb, respectively).

The coffee production plant produced flavored and unflavored whole bean and ground coffee. Full-shift area air samples collected for diacetyl with OSHA Method 1012 and for 2,3-pentanedione with OSHA Method 1016 had highest mean concentrations by location in the grinding/packaging room (103 ppb diacetyl, 63 ppb 2,3-pentanedione), flavoring room (90 ppb diacetyl, 151 ppb 2,3-pentanedione), and the production offices (62 ppb diacetyl, 32 ppb 2,3-pentanedione), which were located within the larger grinding/packaging room. These were followed by mean concentrations in the roasting room (20 ppb diacetyl, 6 ppb 2,3-pentanedione), green bean and finished goods warehouses (11 ppb diacetyl, <3 ppb 2,3-pentanedione), quality control room (8 ppb diacetyl, <3 ppb 2,3-pentanedione), maintenance shop (7 ppb diacetyl, <3 ppb 2,3-pentanedione), and the nonproduction offices (4 ppb diacetyl, <3 ppb 2,3-pentanedione). The flavoring room was under negative pressure with respect to the adjacent grinding/packaging room where unflavored roasted coffee was processed.

Personal sample mean concentrations by location in the coffee plant were highest for employees working in the grinding/packaging room (93 ppb diacetyl, 53 ppb 2,3-pentanedione), flavoring room (80 ppb diacetyl, 122 ppb 2,3-pentanedione), production offices (81 ppb diacetyl, 22 ppb 2,3-pentanedione), all over (59 ppb diacetyl, 39 ppb 2,3-pentanedione), and housekeeping (54 ppb diacetyl, 18 ppb 2,3-pentanedione). These were followed by those in the roasting room (26 ppb diacetyl, 7 ppb 2,3-pentanedione), quality control room (24 ppb diacetyl, 11 ppb 2,3-pentanedione), warehouse

(8 ppb diacetyl, <3 ppb 2,3-pentanedione), and nonproduction offices (7 ppb diacetyl, <3 ppb 2,3-pentanedione).

The mean area concentrations on the grinding/packaging and flavoring room mezzanines, where roasted whole and ground bean storage hoppers were located, were higher than those measured on the main production levels of the rooms. A 15-minute short-term air sample collected at the open hatch of a grinding/packaging room mezzanine hopper holding unflavored ground coffee above an active packaging line measured concentrations of 14,300 ppb diacetyl and 13,800 ppb 2,3-pentanedione. The location of the sample was representative of the proximity of employees' faces as they frequently and momentarily monitored coffee levels in the hoppers throughout their shift.

NIOSH also conducted a small industrywide study at some flavored food production facilities where diacetyl and other food flavorings were added to various food products. Seventy-four personal and 105 area samples were collected for diacetyl using OSHA Method 1013. With one exception where local exhaust ventilation was documented in some locations, no engineering controls were noted in any facility. Of the 179 total samples, 12 had detectable levels of diacetyl (LOD 0.5 – 1.0 ug/sample). The eight area samples ranged from 0.03 to 3.1 ppm, with three samples above 1 ppm (1.1, 2.1 and 3.1 ppm). The four personal samples ranged from 0.06 to 0.6 ppm [Curwin et al. 2015].

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 12 industrial hygiene surveys at various facilities that use (11 facilities) or manufacture (1 facility) formulated flavorings, including flavorings that contain diacetyl [Eastern Research Group 2008a, b, c, d, 2009a, b, c, d, e, 2010a, b, c]. In the first two 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 2-2 below, along with the type of facility and synopsis of controls. Eastern Research Group returned to OSHA G Facility 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 that contained diacetyl. 2,3-Pentanedione was not detected.

2.5.7 Other Exposure Assessments

Pierce et al. characterized diacetyl exposures that could potentially occur in a simulated small coffee shop during the preparation and consumption of unflavored coffee. Mean estimated 8-hour TWA exposure concentrations ranged from 7 ppb to 13 ppb [Pierce et al. 2015].

Gaffney et al. evaluated exposures in a facility that roasts and grinds coffee beans. Results indicated that airborne concentrations of diacetyl and 2,3-pentanedione are similar to concentrations in food flavoring facilities [Gaffney et al. 2015].

Table 2-2. Investigations of facilities using or producing diacetyl

Facility	Product	Controls in place	Diacetyl measurement range
OSHA A [ERG 2008a]	Coffee	Dilution ventilation	ND–54 ppb (TWA) 8 ppb (short-term sample)
OSHA B [ERG 2008b]	Commercial bakery	Dilution ventilation, LEV, process containment	ND–2703 ppb (TWA) ND–1012 ppb (area)
OSHA C [ERG 2008c]	Seasoned snack product	Dilution ventilation; heat extraction for adjacent process	ND
OSHA D [ERG 2008d]	Baked snack food	Secondary heat ventilation for heating and powder dumping	ND–164 ppb (TWA) ND–139 ppb (short-term sample) ND–111 ppb (area)
OSHA E [ERG 2009a]	Sauce production	Engineering controls for other purposes, heat removal, etc.	ND–5.3 ppb (TWA) ND–10.5 ppb (short-term sample) ND–2.4 ppb (area)
OSHA F [ERG 2009b]	Low-calorie cracker	Canopy hoods in heated Production process #1, dilution ventilation	ND–195.7 ppb (TWA) ND–701.1 ppb (short-term sample) ND–13.1 ppb (area)
OSHA G [ERG 2009c]	Buttered popcorn production	Before: Heat extraction hoods, dilution ventilation	24.8–71.2 ppb (TWA) 466.8–2298.7 ppb (short-term sample) 9.1–8660.2 ppb (area)
		After: Dilution ventilation; slot hood at tumbler; modified tank cover; work practice change	< 2.7–< 9.8 ppb (TWA) < 10.4–98.8 ppb (short-term sample) 2.7–5.4 ppb (area)
OSHA H [ERG 2009d]	Sour cream production	Exhaust ventilation for dust	ND–32.4 ppb (TWA) ND–138.6 ppb (short-term sample) ND–4.6 ppb (area)
OSHA I [ERG 2009e]	Ice cream	Controls for other purposes, immediate rinsing, cool temperature dilution ventilation	ND to 1.6 ppb (TWA) ND (short-term sample) ND (area)
OSHA J [ERG 2010a]	Cottage cheese	Dilution ventilation	ND–55.3 ppb (TWA) 32.3–317 ppb (short-term sample) 1.3–32.4 ppb (area)
OSHA K [ERG 2010b]	Food flavor production	Dilution ventilation; hose from tank to floor drain	ND–2,990 ppb (TWA) ND–12,373.1 ppb (short-term sample) 29.1–381.5 (area)
OSHA L [ERG 2010c]	Retail bakery	Dilution ventilation; oven room heat extraction	ND–50.4 ppb (TWA) ND–118.5 ppb (short-term sample) ND–30.9 ppb (area)

ND: not detected
TWA: a sample concentration determined over a full work shift
Short-term sample: a concentration measured for less than a full work shift

References

49 CFR 172.101. Purpose and use of hazardous materials table. Code of Federal Regulations. Washington, DC: U.S. Government Printing Office, Office of the Federal Register.

Ashley K, McKernan LT, Burroughs E, Deddens J, Pendergrass S, Streicher RP [2008]. Analytical performance criteria. Field evaluation of diacetyl sampling and analytical methods. J Occup Environ Hyg *5*(11):D111–116.

Bailey RL, Cox-Ganser JM, Duling MG, LeBouf RF, Martin SB, Bledsoe TA, Green BJ, Kreiss K [2015]. Respiratory morbidity in a coffee processing workplace with sentinel obliterative bronchiolitis cases. Am J Ind Med *58*(12):1235–1245.

Boylstein R [2012]. Identification of diacetyl substitutes at a microwave popcorn production plant. J Occup Environ Hyg *9*(2):D33–34.

Cox-Ganser J, Ganser G, Saito R, Hobbs G, Boylstein R, Hendricks W, Simmons M, Eide M, Kullman G, Piacitelli C [2011]. Correcting diacetyl concentrations from air samples collected with NIOSH Method 2557. J Occup Environ Hyg *8*(2):59–70.

Curwin BD, Deddens JA, McKernan LT [2015]. Flavoring exposure in food manufacturing. J Expo Sci Environ Epidemiol *25*(3):324–333.

Duling MG, LeBouf RF, Cox-Ganser JM, Kreiss K, Martin SB, Bailey RL [2016]. Environmental characterization of a coffee processing workplace with obliterative bronchiolitis in former workers. J Occup Environ Hyg *13(*10):770–781.

Eastern Research Group, Inc. [2008a]. Site visits related to diacetyl and flavorings that contain diacetyl: food production facility A—a coffee roaster. OSHA Docket No. 2008–0046–0068. By Eastern Research Group, Inc. Lexington, MA.

Eastern Research Group, Inc. [2008b]. Site visits related to diacetyl and flavorings that contain diacetyl: food production facility B—a commercial bakery. OSHA Docket No. 2008–0046–0069. By Eastern Research Group, Inc. Lexington, MA.

Eastern Research Group, Inc. [2008c]. Site visits related to diacetyl and flavorings that contain diacetyl: food production facility C—seasoned snack product manufacturing. OSHA Docket No. 2008–0046–0070. By Eastern Research Group, Inc. Lexington, MA.

Eastern Research Group, Inc. [2008d]. Site visits related to diacetyl and flavorings that contain diacetyl: food production facility D—baked snack food production. OSHA Docket No. 2008–0046–0078. By Eastern Research Group, Inc. Lexington, MA.

Eastern Research Group, Inc. [2009a]. Site visits related to diacetyl and flavorings that contain diacetyl: food production facility E—sauce production. OSHA Docket No. 2008–0046–0079. By Eastern Research Group, Inc. Lexington, MA.

Eastern Research Group, Inc. [2009b]. Site visits related to diacetyl and flavorings that contain diacetyl: food production facility F—low calorie cracker production. OSHA Docket No. 2008–0046–0080. By Eastern Research Group, Inc. Lexington, MA.

Eastern Research Group, Inc. [2009c]. Site visits related to diacetyl and flavorings that contain diacetyl: food production facility G—buttered popcorn production (pre-popped). OSHA Docket No. 2008–0046–0081. By Eastern Research Group, Inc. Lexington, MA.

Eastern Research Group, Inc. [2009d]. Site visits related to diacetyl and flavorings that contain diacetyl: food production facility H—sour cream production. OSHA Docket No. 2008–0046–0082. By Eastern Research Group, Inc. Lexington, MA.

Eastern Research Group, Inc. [2009e]. Site visits related to diacetyl and flavorings that contain diacetyl: food production facility I—an ice cream manufacturing plant. OSHA Docket No. 2008–0046–0083. By Eastern Research Group, Inc. Lexington, MA.

Eastern Research Group, Inc. [2010a]. Site visits related to diacetyl and flavorings that contain diacetyl: food production facility J—dairy—cottage cheese production. OSHA Docket No. 2008–0046–0084. By Eastern Research Group, Inc. Lexington, MA.

Eastern Research Group, Inc. [2010b]. Site visits related to diacetyl and flavorings that contain diacetyl: food production facility K—flavor production. OSHA Docket No. 2008–0046–0086. By Eastern Research Group, Inc. Lexington, MA.

Eastern Research Group, Inc. [2010c]. Site visits related to diacetyl and flavorings that contain diacetyl: food production facility L—a retail bakery. OSHA Docket No. 2008–0046–0085. By Eastern Research Group, Inc. Lexington, MA.

Entech Instruments Incorporated [2011]. HDS™ personal monitors. http://www.entechinst.com/pdf/2011%20Updates/Seperate%20Sections/P78-81%20HDS%20Personal%20Monitors.pdf. Date accessed: May 2011.

Gaffney SH, Abelmann A, Pierce JS, Glynn ME, Henshaw JL, McCarthy LA, Lotter JT, Liong M, Finley BL [2015]. Naturally occurring diacetyl and 2,3-pentanedione concentrations associated with roasting and grinding unflavored coffee beans in a commercial setting. Toxicol Rep *2*: 1171–1181.

IATA [2012]. IATA Dangerous Goods Regulations. Montreal, Quebec, Canada: International Air Transport Association.

Kanwal R, Kullman G, Piacitelli C, Boylstein R, Sahakian N, Martin S, Fedan K, Kreiss K [2006]. Evaluation of flavorings-related lung disease risk at six microwave popcorn plants. J Occup Environ Med *48*(2):149–157.

LeBouf RF, Stefaniak AB, Virji MA [2012]. Validation of evacuated canisters for sampling volatile organic compounds in healthcare settings. J Environ Monit *14*(3):977–983.

Martyny JW, Van Dyke MV, Arbuckle S, Towle M, Rose CS [2008]. Diacetyl exposures in the flavor manufacturing industry. J Occup Environ Hyg *5*(11):679–688.

NIOSH [1986]. Hazard evaluation and technical assistance report: International Bakers Services, Inc, South Bend, IN. By McConnell R, Hartle R. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 1985–0171–1710.

NIOSH [1994]. NIOSH manual of analytical methods (NMAM). 4th ed. Schlecht PC, O'Connor PF, eds. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication 94–113 (August, 1994); 1st Supplement Publication 96–135; 2nd Supplement Publication 98–119; 3rd Supplement 2003–154.

NIOSH [2006]. Hazard evaluation and technical assistance report: Gilster-Mary Lee Corporation, Jasper, MO. By Kanwal R, Kullman G, Fedan K, Kreiss K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2000–0401–2991.

NIOSH [2007a]. Hazard evaluation and technical assistance report: Carmi Flavor and Fragrance Company, Inc., Commerce, CA. By Kanwal R, Kullman G. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2006–0303–3043.

NIOSH [2007b]. Hazard evaluation and technical assistance report: Yatsko's Popcorn, Sand Coulee, MT. By Kullman G, Sahakian N. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2006–0195–3044.

NIOSH [2008a]. Evaluating occupational exposures and work practices at Agilex Flavors, Inc. formerly Key Essentials, Inc., Rancho Santa Margarita, CA. A technical assistance report to the California/Occupational Safety and Health Administration. By McKernan L, Dunn K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2006–0361–2.

NIOSH [2008b]. Hazard evaluation and technical assistance report: Gold Coast Ingredients, Inc., Commerce, CA. By Bailey R, McKernan L, Dunn K, Sahakian N, Kreiss K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2007–0033–3074.

NIOSH [2008c]. In-depth survey report: evaluation of engineering controls for the mixing of flavoring chemicals: Gold Coast Ingredients, Inc., Commerce, CA. By Dunn K, Echt A, Garcia A. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for

Occupational Safety and Health, NIOSH EPHB Report No. 322–11a.

NIOSH [2009a]. Hazard evaluation and technical assistance report. Report on an investigation of buttermilk flavoring exposures and respiratory health at a bakery mix production facility, General Mills, Los Angeles, CA. By Day G, Cummings K, Kullman G. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2008–0230–3096.

NIOSH [2009b]. Hazard evaluation and technical assistance report. Respiratory symptoms in workers at three commercial kitchens, Aramark, New York, NY. By Gaughan D, Boylstein R, Iossifova Y, Piacitelli C, Bailey R, Day G. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2008–0125–0126–0127–3093.

NIOSH [2009c]. Hazard evaluation and technical assistance report: Chr. Hansen, Inc., New Berlin, WI. By Sahakian N, Kullman G, Dunn K, Kanwal R. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2007–0327–3083.

NIOSH [2011]. Hazard evaluation and technical assistance report: Lung function (spirometry) testing in employees at a flavorings manufacturing plant—Indiana. By Kreiss K, Piacitelli C, Cox-Ganser J. Morgantown, WV: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2008–0155–3131.

NIOSH [2013a]. Hazard evaluation and technical assistance report: An evaluation of respiratory health at a flavoring manufacturing facility—Kentucky. By Cummings K, Boylstein R, Stanton M, Piacitelli C. Morgantown, WV: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2012–0012–3192.

NIOSH [2013b]. Hazard evaluation and technical assistance report: Evaluation of respiratory concerns at a cream cheese manufacturing facility—New York. By Bailey R, Piacitelli C. Morgantown, WV: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2011–0102–3194.

NIOSH [2013c]. Hazard evaluation and technical assistance report: Evaluation of respiratory concerns at a snack food production facility—New York. By Cummings K, Piacitelli C, Stanton M, Bailey R. Morgantown, WV: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2011–0037–3172.

OSHA [2003]. OSHA Sampling and Analytical Methods: Diacetyl PV2118. http://www.osha.gov/dts/sltc/methods/partial/t-pv2118/t-pv2118.html. Date accessed: June 2009.

OSHA [2008a]. OSHA Sampling and Analytical Methods: Acetoin/Diacetyl 1012. http://www.osha.gov/dts/sltc/methods/validated/1012/1012.html. Date accessed: June 2009.

OSHA [2008b]. OSHA Sampling and Analytical Methods: Acetoin/Diacetyl 1013. http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html. Date accessed: June 2009.

OSHA [2010]. OSHA Sampling and Analytical Methods: 2,3-Pentanedione 1016. http://www.osha.gov/dts/sltc/methods/validated/1016/1016.html. Date accessed: July 2013.

Pierce JS, Abelmann A, Lotter JT, Comerford C, Keeton K, Finley BL [2015]. Characterization of naturally occurring airborne diacetyl concentrations associated with the preparation and consumption of unflavored coffee. Toxicol Rep *2*:1200–1208.

Rodrigues JA, Barros AA, Rodrigues PG [1999]. Differential pulse polarographic determination of alpha-dicarbonyl compounds in foodstuffs after derivatization with o-phenylenediamine. J Agric Food Chem *47*(8):3219–3222.

van Rooy FG, Rooyackers JM, Prokop M, Houba R, Smit LA, Heederik DJ [2007]. Bronchiolitis obliterans syndrome in chemical workers producing diacetyl for food flavorings. Am J Respir Crit Care Med *176*(5):498–504.

White KL [2011]. Correction to microwave popcorn plant diacetyl exposure data. J Occup Environ Hyg *8*(4) (Comment Letter):D25–26.

White KL, Heikkila K, Williams R, Levin L, Lockey JE, Rice C [2010]. Diacetyl exposures at four microwave popcorn plants. J Occup Environ Hyg *7*(4):185–193.

This page intentionally left blank.

3 Effects of Exposure in Employees

Information on the effects on employees' health of exposures to diacetyl and other flavoring compounds comes from case reports and case series and from cross-sectional and longitudinal medical and environmental surveys conducted at several flavoring and food manufacturing facilities (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 compounds 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.

At the time of most of these field investigations, which preceded the California diacetyl regulation implemented in December 2010, little 2,3-pentanedione was being used for artificial butter flavoring. When food manufacturers began to request that diacetyl percentage be less than 1% of flavoring constituents, flavor manufacturers sometimes did not inform their clients of the substitution of 2,3-pentanedione and other diacetyl substitutes [Boylstein 2012; Day et al. 2011; NIOSH 2009b]. Accordingly, populations with 2,3-pentanedione exposure without previous diacetyl exposure are difficult to identify. Thus, illness attributable to 2,3-pentanedione alone has not been studied.

3.1 Obstructive Lung Disease Consistent with Obliterative Bronchiolitis

The most significant health consideration for flavoring-exposed employees is the development of exertional dyspnea or findings consistent with obliterative bronchiolitis (also often called constrictive bronchiolitis, see discussion of terminology). Most textbooks characterize obliterative bronchiolitis as a rare disease with airways obstruction, defined by a decreased FEV_1 and a decreased FEV_1 to FVC ratio on spirometry testing. The magnitude of decline in FEV_1 determines the severity of the disorder. However, three recent case series of biopsy-confirmed obliterative bronchiolitis document that many cases have normal spirometry and, when abnormal, the spirometric pattern can be restrictive, obstructive, or mixed restrictive and obstructive in nature [Ghanei et al. 2008; King et al. 2011; Markopoulou et al. 2002]. Because of the historical assumption that obliterative bronchiolitis is an obstructive disease, the early NIOSH investigations focused on obstructive abnormalities.

Airways obstruction can occur in diseases such as smoking-related COPD (including emphysema and chronic bronchitis) and in asthma. In emphysema, the airways obstruction is usually

Table 3-1. Literature pertinent to flavoring health effects

Reference	Facility NIOSH evaluated	Study type(s), industry	Contribution*
Akpinar-Elci et al. [2004]	G	Case report, MICROWAVE POPCORN MANUFACTURING	Nine former employees at the index plant exhibited moderate to very severe fixed airways obstruction; five of the cases were on lung transplant lists.
Bailey et al. [2015]	R	Public health investigation, FOOD PRODUCTION	Coffee processing employees had excess shortness of breath and 2.7-fold risk of obstructive abnormalities; the group working in both high exposure areas (unflavored coffee grinding/packaging and flavoring room) had lower mean FEV_1/FVC ratio and percent predicted mid-expiratory flow than employees without such exposure.
Cavalcanti et al. [2012]	†	Case report, FOOD PRODUCTION	Four employees at a cookie manufacturing facility developed bronchiolitis within 1 to 3 years of employment.
Centers for Disease Control and Prevention (CDC) [2002]	G	Public health investigation, MICROWAVE POPCORN MANUFACTURING	Eight cases of fixed obstructive lung disease resembling bronchiolitis obliterans among former employees at the index plant resulted in identification of excess risk for mixers compared to packaging employees, with no cases outside of microwave production.
CDC [2007]	†	Case report and public health investigation, FLAVORING MANUFACTURING	Two cases of work-related bronchiolitis obliterans from two different plants resulted in a public health surveillance effort identifying five additional cases of severe fixed obstruction in young, non-smoking employees who worked in flavor compounding or packaging.
CDC [2013]	R	Case report, FOOD PRODUCTION	Two employees in the flavoring room of a coffee roasting plant developed fixed airways obstructive disease and were diagnosed with obliterative bronchiolitis by biopsy.
Halldin et al. [2013]	G	Cohort mortality follow-up, MICROWAVE POPCORN MANUFACTURING	Current and former employees studied by NIOSH in 2000–2003 had a 4-fold increase in mortality coded as chronic obstructive pulmonary disease in an 11-year follow-up through late 2011.
Kanwal et al. [2006]	G, J, K, L, N, O	Summary of six plant surveys, MICROWAVE POPCORN MANUFACTURING	Synthesis of six cross-sectional NIOSH surveys 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 employees near nonisolated tanks of oil and flavorings had higher prevalences of respiratory symptoms and airways obstruction.

See footnotes at end of table.

(Continued)

Table 3-1 (Continued). Literature pertinent to flavoring health effects

Reference	Facility NIOSH evaluated	Study type(s), industry	Contribution*
Kanwal et al. [2011]	G	Intervention study, MICROWAVE POPCORN MANUFACTURING	Ventilation and isolation of flavor mixing at the index plant resulted in one to three orders of magnitude reduction in diacetyl air concentrations in different areas. Employees with high past exposures had stable chest symptoms, decreased mucous membrane and skin symptoms, and higher prevalence of rapid declines in lung function than employees hired after interventions began. These new employees had lower symptom prevalences and higher lung function, demonstrating that intervention resulted in improved health for new employees.
Kim et al. [2010]	B,C†	Cross-sectional industry-wide public health investigation, FLAVORING MANUFACTURING	California flavoring employees had 2.7 times more severe airways obstruction than the general population. Risk factors for the 18 cases with obstruction among 467 employees 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 employees had probable occupational fixed airways obstruction.
Kreiss et al. [2002]	G	Cross-sectional survey, MICROWAVE POPCORN MANUFACTURING	The 117 current employees 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]	L†	Longitudinal survey, MICROWAVE POPCORN MANUFACTURING	Study of 765 employees 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 was associated with an odds ratio of 9.2 for obstruction.
NIOSH [1986]	A	Cross-sectional survey, FLAVORING MANUFACTURING	Two young employees with no known risk factors developed severe, fixed obstructive lung disease suggestive of bronchiolitis obliterans within 1 year of employment.
NIOSH [2003a]	N	Cross-sectional survey, MICROWAVE POPCORN MANUFACTURING	Elevated prevalence of airways obstruction when compared to national rates; all observed obstruction was fixed.

See footnotes at end of table.

(Continued)

Table 3-1 (Continued). Literature pertinent to flavoring health effects

Reference	Facility NIOSH evaluated	Study type(s), industry	Contribution*
NIOSH [2004a]	K	Cross-sectional survey, MICROWAVE POPCORN MANUFACTURING	A study of 157 employees in a plant having a mixing room employee previously diagnosed with fixed obstructive lung disease consistent with bronchiolitis obliterans found abnormal lung function in 6 of 13 mixers: 3 had fixed airways obstruction, and 3 had spirometric restriction.
NIOSH [2004b]	L	Cross-sectional survey, MICROWAVE POPCORN MANUFACTURING	A survey of 205 employees 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 employees with fixed airways obstruction and normal diffusing capacity.
NIOSH [2006]	G	Eight cross-sectional surveys, MICROWAVE POPCORN MANUFACTURING	Studies of 373 current employees over 2.75 years at the index plant determined that inhalation of butter flavoring compounds is a risk for occupational disease. Comparisons of employees hired before and after implementation of exposure controls document declines in the prevalence of eye, nose, and throat irritation. Large decreases in FEV_1 (> 300 mL or 10%) were observed in 4 of 9 mixers; one young mixer lost 2,800 mL over 2.75 years.
NIOSH [2007a]	B	Two cross-sectional surveys, FLAVORING MANUFACTURING	Two former employees and one current employee who made powdered flavorings (of 18 with current or previous production experience) had severe fixed obstructive lung disease consistent with bronchiolitis obliterans. Within months of the survey, one of these employees was diagnosed with bronchiolitis obliterans following biopsy.
NIOSH [2007b]	M	Cross-sectional survey, FOOD PRODUCTION	All three employees in a popcorn popping business developed symptoms of airways disease during their tenure; all were lifetime nonsmokers. One of the employees had significant reversible airways obstruction with some clinical evidence suggesting possible bronchiolitis obliterans in addition to asthma.

See footnotes at end of table.

(Continued)

Table 3-1 (Continued). Literature pertinent to flavoring health effects

Reference	Facility NIOSH evaluated	Study type(s), industry	Contribution*
NIOSH [2008]	C	Two cross-sectional surveys, FLAVORING MANUFACTURING	One of 14 employees with production experience had severe fixed airways obstruction (subsequently confirmed as bronchiolitis obliterans), and an additional production employee developed mild fixed obstruction following the loss of 1 liter in FEV_1 during a 4.5-month screening interval.
NIOSH [2009b]	E	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 employees tested. Participants had higher than expected rates of shortness of breath, physician-diagnosed asthma, and a restrictive pattern on spirometry (four cases ranging from mild to moderately severe), compared to U.S. adults. Some participants reported symptoms with a work-related pattern.
NIOSH [2009c]	F	Three cross-sectional surveys, FOOD PREPARATION	Studies of employees 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 employees did not appear to be work-related.
NIOSH [2009d]	D	Cross-sectional survey, FLAVORING MANUFACTURING	This study of 34 employees found that bacterial products employees had higher prevalences of work-related eye symptoms and post-hire skin problems than flavoring employees; both groups reported lower respiratory symptoms related to the substances they handled at work. One employee was identified with fixed airways obstruction and two employees with restriction on spirometry.

See footnotes at end of table.

(Continued)

Table 3-1 (Continued). Literature pertinent to flavoring health effects

Reference	Facility NIOSH evaluated	Study type(s), industry	Contribution*
NIOSH [2011] Kreiss [2014]	I	Public health investigation, FLAVORING MANUFACTURING	Review of company-supplied spirometry tests identified 39 (37%) employees with evidence of some abnormality (either the most recent spirometry test showed restriction or obstruction; and/or longitudinal spirometry showed excessive decline over time, with most recent spirometry values still within the normal range). Employees currently in areas with higher potential for flavorings exposure were 5.8 times more likely to have abnormal declines in FEV_1 than employees in other areas.
NIOSH [2013] Cummings et al. [2014]	Q	Cross-sectional survey, FLAVORING MANUFACTURING	A survey of 367 employees at a plant that used thousands of chemicals found that employees that spent 1 hour or more daily in the production area had higher prevalences of spirometric abnormalities and low diffusing capacity. Employees with 7 or more years of tenure had lower mean values of percent predicted FEV_1, FVC, and diffusing capacity.
van Rooy et al. [2007]	†	Case report, Cross-sectional survey, DIACETYL MANUFACTURING	Four cases of bronchiolitis obliterans syndrome ([BOS] previously attributed to chronic obstructive pulmonary disease [COPD] or asthma) were identified in screening of 103 process operators in a retrospective cohort of production plant employees.
van Rooy et al. [2009]	†	Cross-sectional survey, DIACETYL MANUFACTURING	Excess respiratory symptoms and asthma indices occurred among 175 production plant employees compared to an internal reference group and a general population sample, with evidence of diacetyl exposure-response for FEV_1.

* The health effects listed use the terminology stated in the original report or publication (e.g., fixed obstruction, bronchiolitis obliterans, bronchiolitis obliterans syndrome).
† Referenced publication includes site(s) that NIOSH did not visit.

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 employees who have developed obstructive lung disease while exposed to diacetyl and other flavoring compounds have had fixed airways obstruction. Additional medical tests in severely affected employees have generally revealed findings consistent with the irreversible obstructive lung disease obliterative bronchiolitis (discussed in detail in section 3.1.2). Serial lung function testing with spirometry indicates that affected employees can experience very rapid lung function declines.

Obstructive lung disease in employees exposed to diacetyl and other flavoring compounds was first reported in employees 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 employees, 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 employees 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 thought to suffer from this pathologic condition based on clinical findings, 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 bronchiolitis obliterans organizing pneumonia (BOOP) 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 and the European Respiratory Society 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, and aspiration pneumonitis. Proliferative bronchiolitis is generally responsive to corticosteroid medications and is usually reversible [King and Kinder 2008].

Obliterative bronchiolitis (also referred to as constrictive bronchiolitis obliterans [ATS and ERS 2002], constrictive 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 usually show airways obstruction and hyperinflation [King and Kinder 2008], but biopsy-confirmed cases may have normal or restrictive spirometry [Ghanei et al. 2008; King et al. 2011; Markopoulou et al. 2002]. 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 obliterative 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 obliterative bronchiolitis; scans with both inspiratory and expiratory views are helpful because expiratory views are important in assessing air trapping [King 2003]. Identification of the obliterative bronchiolitis lesion on lung biopsy may be difficult because of 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]. The diagnosis is a multidisciplinary one involving a team with clinical, radiologic, and histopathologic expertise; HRCT evidence often replaces the need for surgical lung biopsy [King and Kinder 2008]. In comparison to proliferative bronchiolitis, obliterative bronchiolitis is generally unresponsive to corticosteroid medications and often progresses to more severe disease [King and Kinder 2008], although progression after exposure cessation is not characteristic of flavoring-related disease consistent with obliterative bronchiolitis [Akpinar-Elci et al. 2004].

As mentioned previously and discussed in detail in the next section (3.1.2), the medical evaluations of employees who have developed lung disease during exposure to diacetyl and other flavoring compounds have generally revealed findings consistent with obliterative bronchiolitis. Because of concerns for patient welfare and the invasive nature and imperfect sensitivity of lung biopsy for diagnosing obliterative bronchiolitis, most patients have been diagnosed based upon clinical findings. Despite the small number of lung biopsies conducted, findings consistent with obliterative bronchiolitis 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 obliterative 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 obliterative bronchiolitis 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 obliterative bronchiolitis 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. The syndrome refers to graft deterioration secondary to persistent airflow obstruction as defined by pulmonary function changes with or without histolopathologic 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 obliterative bronchiolitis resulting from chemical injury and diagnosed using clinical criteria with or without biopsy [Akpinar-Elci et al. 2004; Ghanei et al. 2004a; van Rooy et al. 2007].

Because the terminology used in the peer-reviewed literature of flavorings-exposed employees 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 sometimes 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 obliterative bronchiolitis in microwave popcorn employees 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 employees (index cases) of a microwave popcorn plant (index Facility G)[CDC 2002]. NIOSH reviewed medical records for these employees and in November 2000 conducted a medical survey of current and former employees of this plant. Survey results and medical records review for the eight index cases and a current employee with lung disease showed several findings consistent with obliterative bronchiolitis. All cases had moderate to very severe airways obstruction (FEV_1s 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. 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 employees 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 employees had been working in the room where butter flavorings, salt, and colorings were combined with heated soybean oil. The other four employees had been working in the adjacent room where the oil and flavoring mixture was combined with kernel popcorn in microwavable bags (packaging area). None of these employees were initially diagnosed by their personal physicians as having obliterative bronchiolitis.

Initial diagnoses received by these employees included pneumonia, asthma, emphysema, bronchitis, COPD, hay fever, and sinusitis. Five of the employees had minimal smoking history. All nine employees had been prescribed oral corticosteroids, but none had improvement in lung function. Five of the employees 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 (Facility G) 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 employees, 117 (87%) completed the questionnaire, and 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 such as plain kernel popcorn packaging, offices, warehouse, and outside receiving. The prevalences of respiratory and systemic symptoms, mucous membrane irritation, and skin irritation were higher among employees 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 employees who underwent spirometry, 21 had airways obstruction, 3.3 times higher than expected. Airways obstruction in nonsmokers was 10.8 times higher than expected, and only two employees with airways obstruction had a significant response to administered bronchodilator. Five of six employees in the quality control (QC) laboratory had airways obstruction; these employees popped up to 100 bags of microwave popcorn in microwave ovens per employee 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 employees 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 (Facility G) in November 2000 [Kanwal et al. 2011; NIOSH 2006]. The predominant VOC in the air of the plant was the butter flavoring compound 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 relative humidity and temperature measurements used for correction were available from in-facility area-specific and shift-specific measurements during all sampling, and sample-specific days to extraction were supplied by the laboratory. 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 it was 0.9 ppm. The much higher prevalence of airways obstruction in QC employees, despite much lower average

air concentrations of diacetyl, may reflect an enhanced risk of peak flavoring exposures when microwaved bags of popcorn product were opened; peak exposures were also likely present in maintenance employees and mixers. 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 employees 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 (corrected for underestimation by NIOSH Method 2557), the prevalence of any airways obstruction on spirometry was 14.3% in the lowest exposure quartile, 6.7% in the next lowest quartile, and 27.6% in the highest two exposure quartiles (statistically significant; *P* for trend = 0.04). The prevalences of abnormal spirometry, whether obstructed or restricted or mixed, by quartile were 21.4% for cumulative exposures < 0.82 ppm-yr; 16.7% for cumulative exposures between 0.82 and < 6.4 ppm-yr; 34.5% for cumulative exposures between 6.4 and < 19.2 ppm-yr; and 37.9% for cumulative exposures > 19.2 ppm-yr (statistically significant; *P* for trend = 0.04). Lung function as indicated by the average percent of predicted FEV_1 on spirometry was 93.5%, 95.8%, 86.5% and 84.3% in the lowest to highest quartiles (*P* for trend = 0.03) [Kreiss et al. 2002].

3.1.2.3 Findings of index plant follow-up surveys

NIOSH conducted seven follow-up medical and eight follow-up environmental surveys at the index microwave popcorn plant (Facility G) from 2001 to 2003 [Kanwal et al. 2011; NIOSH 2006]. These surveys were conducted to follow employee symptoms and lung function over time as exposures decreased with the implementation of engineering controls. NIOSH recommended a respiratory protection program for mixing room employees 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 engineering controls to decrease air concentrations of flavoring compounds 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 in which 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 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 Facility G from November 2000 to August 2003, NIOSH compared health outcomes in microwave popcorn production employees hired after the implementation of exposure controls to health outcomes in employees who had been working at the plant prior to the implementation of controls [Kanwal et al. 2011]. For these analyses, investigators classified employees according to their hire date as follows: "Group 1" consisted of employees 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 employees who started work at the plant after the November 2000 survey (i.e., after exposure controls were implemented and exposures had declined). Because of a high turnover rate among employees 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 employees 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 employees who participated in more than one survey worked in the packaging area. Therefore, for all Group 2 packaging area employees 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 employees' first surveys, packaging area employees 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 employees in Group 1. All these 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 employees [Kanwal et al. 2011]. Of interest is that 47% of all employees with abnormal spirometry tested by NIOSH (in Groups 1 and 2) were asymptomatic.

NIOSH conducted a mortality study on Facility G employees based on Social Security Administration vital status determination as of November 30, 2011 [Halldin et al. 2013]. The cohort consisted of employees with potential flavoring exposure: 356 current employees who had participated in any of the eight NIOSH cross-sectional medical surveys from November 2000 through August 2003 and 155 former employees tested by NIOSH at the county health department during that time period. There were 15 decedents altogether, not significantly different from the 17.39 expected. However, there were five COPD-associated multiple causes of death (International Classification of Diseases [ICD]-10 codes

J40-J44) coded among four decedents, for a standardized mortality ratio (SMR) of 4.3 (95% confidence interval [CI] 1.40–10.04). There is no specific ICD-10 code for obliterative bronchiolitis, so it is likely that death from the condition would be coded using a COPD classification code. Consistent with this, the specific code J44 "other COPD" was assigned as a multiple cause of death for the four decedents (0.98 expected; SMR = 4.10, 95% CI 1.12–10.49). Three of the four COPD-coded deaths occurred among former employees and employees employed before the company began to implement interventions to reduce diacetyl exposure (Group 1 above).

3.1.2.4 Other NIOSH microwave popcorn health hazard evaluations

NIOSH conducted HHEs that included cross-sectional medical and environmental surveys at five other microwave popcorn plants (Facilities J, K, L, N, and O) from 2001 to 2003 [NIOSH 2003a, b, c, 2004a, b]. These plants and the index plant (Facility G) 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 evaluated by NIOSH were as follows:

(1) At each plant, one to three employees 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) Employees on the packaging lines operated the packaging machines and facilitated the placement of the finished product into cartons and boxes.

In most plants, QC employees popped product in microwave ovens that were usually located in a separate QC laboratory. Other employees were located in warehouse and office areas. In separate areas of some plants, employees 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 (Facilities J and O) had fewer than 15 employees, one or two mixing tanks, and one packaging line.

(2) One medium-sized plant (Facility N) had approximately 50 employees, one mixing tank, three holding tanks for heated oil and butter flavoring mixtures, and three packaging lines.

(3) The three largest plants (Facilities G, K, and L) had more than 100 employees, 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 employees with findings consistent with bronchiolitis obliterans at the index microwave popcorn plant, employees with fixed airways obstruction and air trapping on HRCT scans consistent with obliterative bronchiolitis 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; NIOSH 2003b, 2004a, b]. Like the index plant, the medium-sized plant had affected packaging area employees. At both of these plants, packaging area employees worked near tanks of heated oil and butter flavorings [NIOSH 2003a, 2006]. The biopsies of three of the six employees 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 were aggregated with the data from the surveys at the other plants. Compared to employees who had never worked as mixers, employees 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 employees at plants where tanks of heated oil and butter flavorings were isolated from the packaging lines, packaging area employees 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 employees 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 employees 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 employees at plants where tanks were isolated. The prevalences of other respiratory symptoms were similar in both groups. The findings across the six plants suggested that those employee groups with peak exposures, sometimes with relatively low average exposures, had higher prevalences of chest symptoms or pulmonary function abnormalities than those employees without intermittent high exposures [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, Facility L, 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 obliterative bronchiolitis. 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 employees 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 employees and 21 employees with a history of asthma that began prior to employment and who were taking asthma medications. The investigators classified employees into five groups for data analyses: (1) non-mixers (i.e., employees 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) with an assigned protection factor of 25 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 employees 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-yrs 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 a mixer before the implementation of mandatory PAPR use was associated with an 8-fold increased risk of airways obstruction (95% 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-yrs 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 employees with a progressive increase or decrease in FEV_1 of greater than 8% or 330 mL over 12 months among employees 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 employees 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. Analyses of 6 years of spirometric follow-up of these four plant cohorts are pending.

3.1.2.6 Field studies at flavoring manufacturing plants

Employees at several flavoring manufacturing plants have developed severe fixed airways obstruction and other findings consistent with obliterative bronchiolitis [Kanwal 2008]. The first known publicly available report of bronchiolitis obliterans in flavoring manufacturing employees is a 1986 report of a NIOSH HHE at Facility A that manufactured flavors for the baking industry [NIOSH 1986]. 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 employee 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 employees. NIOSH reported that one employee had a "mild" response to bronchodilators and the other had a "minimal" response. Neither employee 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 employees, and chest x-rays were normal or showed hyperinflation. NIOSH concluded that, even without pathological confirmation, the clinical picture was more compatible with bronchiolitis obliterans than with emphysema. One of the two employees 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 employee left the job when he was identified with severe fixed airways obstruction 5 months after starting work at the plant in the same job. Two current mixers with 5 to 6 years of 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 at Facility A, mixers produced flavors by mixing liquid flavor compounds into dextrose and corn starch powder in large blenders. This included using both 300-pound and 500-pound capacity "day mixers" (ribbon blenders), and a 1,500-pound capacity Littleford Mixer [NIOSH 1986]. Employees used approximately 200 Food and Drug Administration (FDA)-approved flavor compounds 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 employee to develop severe fixed airways obstruction had started work. Management had required employees to wear respirators when weighing or adding the flavors or base ingredients to the mixers. However, employees 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 1986]. Air sampling for specific flavoring compounds was not conducted.

A cluster of cases consistent with obliterative bronchiolitis among production employees at a flavoring manufacturing company was reported by Dr. James Lockey at the 2002 American Thoracic Society International Conference [Lockey et al. 2009]. After identification of an index case of biopsy-documented bronchiolitis obliterans at this plant, a survey of the workforce identified an additional four employees with clinical findings consistent with obliterative bronchiolitis. All five employees with these findings had normal spirometry tests at the start of employment. These employees went on to develop moderate to severe fixed airways obstruction. For 4 to 5 years after cessation of exposure to flavoring compounds, the affected employees had no further declines in their lung function.

In 2007, the California Department of Public Health reported that seven flavoring manufacturing employees 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 (Facilities B and C) [NIOSH 2007a, 2008]. Facility B produced liquid and powdered flavorings; powdered flavorings were produced by combining liquid flavoring compounds such as diacetyl with powder ingredients in ribbon blenders. Out of a workforce of 36 at the time of the NIOSH survey, 12 worked in the flavoring production room. Before July 2006, management provided production employees with 3M® N95 filtering-facepiece respirators for voluntary use. In 2005, a 42-year-old production employee who had worked for 7 years primarily making powdered flavorings developed cough and progressive shortness of breath. Medical tests conducted by this employee'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 computed tomography (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 employee favored a diagnosis of bronchiolitis obliterans over hypersensitivity pneumonitis. This employee 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 employee again showed severe fixed airways obstruction (FEV_1 of 0.54 liters; 21% of predicted). Another former employee and a current employee 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 employee and 0.78 liters (23% of predicted) for the current employee. The current employee with severe airways obstruction reported a past history of asthma but 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 survey 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 employee was found to have mild restriction on spirometry; the rest of the medical survey participants (31 of 36 current employees and three former employees) had normal spirometry tests [NIOSH 2007a].

NIOSH conducted an HHE at a second flavoring manufacturer (Facility C) over several visits to the plant from October 2006 to July 2007 [NIOSH 2008]. This plant produced liquid and powdered flavorings (encapsulated and nonencapsulated powders) and colors. Nonencapsulated powdered flavorings were produced by combining liquid flavoring compounds such as diacetyl with powder ingredients in ribbon blenders. Encapsulated powdered flavorings were 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 survey, 12 were production employees. Forty-one employees participated in the first NIOSH medical survey conducted from October 30, 2006, to November 1, 2006. Of 41 employees tested, 3 had abnormal spirometry: a laboratory/QC employee had mild restriction, a flavoring production employee had borderline obstruction, and an employee in the warehouse with several years of experience in flavoring production had severe fixed airways obstruction. This last employee 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 employee 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 employee 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%).

NIOSH performed an HHE in 2007 that included a medical and environmental survey at a flavoring manufacturer (Facility D) 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 employees 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 employees, 15 worked in jobs where they could potentially be exposed to flavoring-related compounds

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

In 2012, NIOSH conducted a cross-sectional medical survey at a flavoring company (Facility Q) in Kentucky in which two former employees had received physician diagnoses of obliterative bronchiolitis [Cummings et al. 2014; NIOSH 2013]. Of 357 employees with spirometry, 13 had obstruction (of whom 2 of 10 responded to bronchodilator), 15 had restriction, and 2 had mixed obstruction and restriction. The prevalences of abnormal spirometry were not elevated in relation to NHANES III expected rates, adjusted for age, sex, race/ethnicity, smoking status, and body mass index. However, participating employees had statistically significant excesses of wheeze in the last 12 months, sinusitis or sinus problems in the last 12 months, phlegm on most days for three consecutive months during the year, a diagnosis of hay fever, a lifetime diagnosis of asthma, and current asthma, when compared to the U.S. adult population. Shortness of breath was twice as common in those with 7 or more year's tenure, and remained significant in a model adjusted for age and smoking status. Work-related breathing trouble, wheeze, nasal symptoms, sinusitis, eye symptoms, and cough were all statistically significantly increased in employees currently using flavoring compounds compared to remaining employees, and these work-related symptoms remained significantly associated with flavoring compound use in models adjusted for age and smoking status. Participating employees who spent an hour or more daily in production areas had twice the prevalence of any spirometric abnormality and three times the prevalence of low diffusing capacity than other participants. Mean lung function parameters (expressed as percent predicted) were significantly lower in participants with tenure of 7 or more years and those who spent one or more hours daily in production areas. Differences in lung function could not be explained by age, smoking status, or employment at another flavoring plant, and persisted in analyses stratified by ever having been in production. The association of symptoms and lung function parameters with exposure indices suggested that they resulted from workplace exposures. Diacetyl was not used in the plant during the NIOSH air sampling, but 2,3-pentanedione was detected in two air samples collected with evacuated canisters in a liquid compounding room where fruit and cheese flavor recipes were being prepared.

3.1.2.7 Lung disease in flavoring manufacturing employees

The California Department of Public Health provided information on a flavoring production

employee who developed bronchiolitis obliterans while working at another California flavoring plant [California Department of Public Health 2007; CDC 2007]. This employee primarily prepared powdered flavorings by pouring "diacetyl and other liquid ingredients through a hole on the blender lid." 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, Cal/OSHA and the California Department of Public Health developed a lung disease prevention program for employees of California flavoring manufacturing plants. In analyses of aggregated medical surveillance data (questionnaire and spirometry results) from 467 employees at 16 companies who had usable questionnaires and acceptable spirometry tests, 18 employees (3.9%) from six companies with 315 participating employees had airways obstruction [Kim et al. 2010]. This prevalence was similar to that expected in comparison to national data from NHANES III [CDC 1996]. 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 employees less than 40 years old (95% CI 5.1–44.1). Sixteen obstructed cases worked in four companies using ≥ 800 pounds of diacetyl annually compared to two obstructed cases in companies using less diacetyl (prevalence of 5.3% versus 1.2%), for an odds ratio (OR) of 4.5 (95% CI 1.03–19.9). The prevalence of obstruction in employees currently doing any production task was 4.5% compared to 2.0% in production support employees (laboratory technicians/scientists, quality control technicians, maintenance/repair employees, warehouse employees, and truck drivers) and 2.3% in office employees. Of the 18 employees 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 employees with moderate or worse obstruction than in employees with mild obstruction (1.5 versus 9.0 years; $P = 0.02$). Half of the 18 employees with obstruction reported no chest symptoms (five of six employees with mild obstruction and four of seven with moderate obstruction). Of the 13 with documented postbronchodilator spirometry, 12 had fixed obstruction (including all four with severe or very severe obstruction). Of the 12 of 18 with obstruction who had medical evaluation results submitted to the California Department of Public Health, eight were diagnosed by their physicians to have either bronchiolitis obliterans (one biopsy-confirmed) 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 employee surveillance data were presented above in the descriptions of two NIOSH HHEs at California flavoring plants (Facilities B and C).

3.1.2.8 Lung disease in diacetyl production employees

Lung disease consistent with obliterative bronchiolitis was reported among employees 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 employees 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. Employees 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 employees were found to have fixed airways obstruction. One of these four employees (with a predicted FEV_1 of 72%) refused further evaluation. FEV_1 percent predicted in the other three employees, all process operators, ranged from 35% to 42%. All three employees had evidence of air trapping on HRCT scan expiratory views. One of these three employees underwent a thoracoscopic lung biopsy that did not show evidence of obliterative bronchiolitis. Two of these three employees were nonsmokers who had initially been diagnosed with COPD; the third employee (with a 14 pack-year smoking history) had initially been diagnosed with COPD and asthma. Two of these three employees 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 employee developed shortness of breath at age 52, 14 years after starting work. A fourth employee (process operator; nonsmoker) with severe fixed airways obstruction and findings compatible with obliterative bronchiolitis on HRCT scan was identified among survey nonparticipants after the survey. During production of diacetyl, employees 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 employees 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]. Regarding diacetyl exposures, 26 area samples (82–219 minutes) and 4 personal task-based samples (33–90 minutes) were taken between 1995 and 2003. Many jobs were not sampled. These data were insufficient for quantitative risk assessment over the period of plant operation from 1960 to 2003.

The investigators who evaluated the workforce of the diacetyl-producing plant in the Netherlands compared respiratory symptom and asthma prevalence among male employees 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

employees 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 white male diacetyl plant employees 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 employees have developed findings consistent with obliterative bronchiolitis indicates that employees' exposures in these plants may have been similar to employees' 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. Employees' real-time diacetyl exposures during the packaging of these powders were as high as 204 ppm. At a second flavoring plant [NIOSH 2008], 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 an employee 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 employees 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 (corrected 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 (Facility G), 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 findings consistent with obliterative bronchiolitis, TWA diacetyl exposures from personal samples were 0.31 ppm, 0.69 ppm, and 1.33 ppm [NIOSH 2003b, 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].

3.1.2.9 Other food production case reports

In addition to cases consistent with obliterative bronchiolitis in flavoring manufacture, diacetyl manufacture, and microwave popcorn production, case reports have surfaced in other food production industries in which flavorings are introduced into food products. In cookie manufacture with artificial butter flavoring in Brazil, four cases of bronchiolitis were described in young men, aged 24 to 27 years, who had worked between 1 and 3 years handling flavorings in preparation of cookie dough [Cavalcanti et al. 2012]. One of the four had confirmation of bronchiolitis obliterans on open lung biopsy, and the remaining three were diagnosed on the basis of consistent chest symptoms (cough, progressive dyspnea,

and wheezing); moderate to severe mixed obstructive and restrictive spirometry; abnormal chest CT findings of lung hyperinflation, air trapping, bronchial thickening, and mosaic perfusion; and persistence of spirometric findings in 4 years of follow up. Two cases had partial response to bronchodilators and one case had ground-glass opacity.

In a coffee production plant, two cases have biopsy confirmation of obliterative bronchiolitis among employees with artificial flavorings exposure in the production of roasted coffee beans and ground coffee [CDC 2013]. An additional three cases from the same plant were reported by Dr. Jeffrey Levin in an abstract at the 2013 American Thoracic Society International Conference. In 2012, NIOSH conducted an HHE at this coffee production plant involving 75 current employees (88% participation)[Bailey et al. 2015]. Excluding the five sentinel former employees (all never-smokers under age 42), standardized morbidity ratios were elevated 1.6-fold for shortness of breath and 2.7-fold for obstructive spirometric abnormalities. The sum of diacetyl and 2,3-pentanedione air concentrations were equivalent in the flavoring room and in the grinding/packaging area of unflavored coffee. The subgroup of employees who currently worked in both coffee flavoring and grinding/packaging of unflavored coffee had significantly lower mean FEV_1/FVC ratio and percent predicted mid-expiratory flow than employees without such exposure. In addition to the sentinel former employee cases, six current employees had abnormalities suggestive of obliterative bronchiolitis and five additional employees had suspect work-related asthma. NIOSH investigators could not separate risks of employees in unflavored coffee grinding/packaging from risks of flavored coffee production because most employees' exposures were similar during their work tenure.

3.2 Restrictive Spirometry in Flavoring-exposed Workforces

NIOSH work on flavoring-related lung disease concentrated on obstructive spirometric abnormalities between 2000 and 2008 because the classic textbook description of obliterative bronchiolitis described an obstructive disease. NIOSH included employees with mixed obstructive and restrictive spirometry among the obstructed because NIOSH assumed that air trapping explained their decreases in forced vital capacity. Had NIOSH added employees with mixed obstructive and restrictive disease to those with pure spirometric restriction, NIOSH would have had excesses of restriction similar to those of obstruction in comparison to general population prevalences in some field investigations. NIOSH now has evidence from several investigators outside of the flavoring lung disease field that the clinical spectrum of biopsy-confirmed obliterative bronchiolitis includes both restrictive spirometry and normal spirometry, as well as those with fixed obstructive spirometry [Ghanei et al. 2008; King et al. 2011; Markopoulou et al. 2002]. NIOSH summarizes the evidence concerning spirometric restriction in flavoring-exposed employees in this section.

Spirometric restriction is defined as a FVC below the lower limit of normal and an FEV_1/FVC ratio that is normal. Lung diseases involving scarring (fibrosis), and inflammation of the interstitium or alveolar spaces commonly are accompanied by spirometric restriction. Examples of these lung diseases are hypersensitivity pneumonitis, pneumoconioses, and BOOP in which bronchiolar changes extend into the alveolar spaces. Non-pulmonary causes of spirometric restriction are poor effort or incomplete exhalation maneuvers, obesity, and neuromuscular weakness. Low lung volumes such as total lung capacity

and low residual volume support pulmonary causes of spirometric restriction, but normal lung volumes do not rule out lung pathology accounting for spirometric restriction [Boros et al. 2004].

The proportions of abnormal spirometry that were restrictive in the three case series of biopsy-documented constrictive bronchiolitis are instructive. In dyspneic U.S. soldiers, 3 of 38 soldiers had restriction (2 with low diffusing capacity), 2 had obstruction (1 with low diffusing capacity), and one had mixed restriction and obstruction (with low diffusing capacity). The remaining soldiers had normal spirometry and lung volumes, although 19 had low diffusing capacity [King et al. 2011]. Of 15 cases of chronic dyspnea and cough following sulfur mustard exposure 20 years previously, 13 had normal spirometry, one had restriction, and one had obstruction; all had pathologic evidence of bronchiolar disease. The cases with biopsy-documented constrictive bronchiolitis all had normal spirometry, and the two with the abnormal spirometry had chronic cellular bronchiolitis [Ghanei et al. 2008]. Of 19 cases of biopsy-documented obliterative bronchiolitis, six had normal spirometry (although 2 had isolated gas trapping), 11 had obstruction, one had restriction, and one had a mixed pattern [Markopoulou et al. 2002]. This last case series originated from a clinical referral center without common exposures. These pathologic case series suggest two conclusions. First, abnormal spirometry is insensitive to pathologic obliterative bronchiolitis that results in symptoms warranting clinical evaluation. Second, the finding of restriction in populations with cases of fixed airways obstruction consistent with obliterative bronchiolitis is likely to be part of the spectrum of obliterative bronchiolitis, although the differential diagnosis in individual employees requires investigation.

3.2.1 Index Plant Findings Regarding Restriction

Among the former employees who developed findings consistent with obliterative bronchiolitis while working at the index microwave popcorn plant (Facility G), lung function tests in one employee showed a reduced total lung capacity and reduced residual volume in addition to airways obstruction. These reduced lung volumes indicate that this employee had restrictive lung disease as well as airways obstruction [Akpinar-Elci et al. 2004]. This former employee also had a low carbon monoxide diffusing capacity and was unusual among the former employee cases in having some reversibility after ceasing employment at the microwave popcorn [Akpinar-Elci et al. 2004].

In the first cross-sectional survey of the index plant (Facility G), 10 of 116 employees had isolated abnormal FVC, of whom 7 had low total lung capacity; 11 employees had isolated airways obstruction. An additional 10 employees had both low FVC and airways obstruction, for a total of 21 of 116 employees having any restrictive spirometric pattern. None of those with any restriction had radiologic interstitial abnormalities. When the prevalence of any restrictive abnormality was examined by cumulative exposure quartile (using exposure estimates corrected for humidity and time to extraction), a trend for exposure response relationship was evident: From lowest to highest exposure quartile, the prevalence of any restriction was 10.7%, 13.3%, 20.7%, and 24.1% (P = 0.08). During follow up of these plant employees, one employee with rapidly falling pulmonary functions in a restrictive pattern underwent open lung biopsy. The pathology report documented caseating lung granulomas around airways, but grossly normal areas of lung were not sampled for examination of possible obliterative bronchiolitis. Cultures and stains for microorganisms did not yield an infectious etiology, and the physician concluded

Table 3-2. Distribution of spirometric abnormalities in flavoring-exposed employees by facility, reference, facility type, and type of spirometric abnormality

Facility NIOSH evaluated	Reference	Facility type	Current employees tested	Spirometric abnormalities		Restrictive abnormalities		Obstructive abnormalities	Mixed obstructive and restrictive abnormalities
				N	%	N	%*		
G	Kreiss et al. [2002]	Microwave popcorn	116	31	26.7	10	32.3	11	10
N	NIOSH [2003a]	Microwave popcorn	35	7	20.0	2	28.6	2	3
K	NIOSH [2004a]	Microwave popcorn	86	13	15.1	7	53.8	3	3
L	NIOSH [2004b]	Microwave popcorn	205	29	14.1	10	34.5	12	7
G	NIOSH [2006]	Microwave popcorn	368	75	20.4	36	48.0	24	15
B	NIOSH [2007a]	Flavor manufacturer	34	2	5.9	1	50.0	0	1
C	NIOSH [2008]	Flavor manufacturer	41	3	7.3	1	33.3	1	1
D	NIOSH [2009d]	Flavor manufacturer	28	3	10.7	2	66.7	0	1
E	NIOSH [2009b]	Food production	22	4	18.2	4	100.0	0	0
F	NIOSH [2009c]	Food preparation	104	20	19.2	15	75.0	4	1
B,C†	Kim et al. [2010]	Flavor manufacturer	467	59	12.6	41	69.5	10	8
I	NIOSH [2011]	Flavor manufacturer	106	34	32.1	30	88.2	3	1
G	Halldin et al. [2013]	Microwave popcorn	356	57	16.0	27	47.4	16	14
			155‡	50	32.3	23	46.0	10	17
Q	NIOSH [2013]	Flavor manufacturer	357	30	8.4	15	50.0	13	2
R	Bailey et al [2015]	Food production	69	7	10.2	2	2.9	5	0

*Percentage of abnormal pulmonary function tests (PFTs) classified as restriction

†Referenced publication includes site(s) that NIOSH did not visit

‡Former employees

that the abnormalities were related to occupational flavoring exposures [Kreiss 2012].

3.2.2 NIOSH Findings of Restrictive Spirometry at Other Microwave Popcorn Plants

Most microwave popcorn plant populations surveyed cross-sectionally by NIOSH had similar proportions of restrictive, obstructive, and mixed abnormalities among those employees with abnormal pulmonary functions (Table 3-2). In the three large microwave popcorn plants (Facilities G, K, and L), the restrictive proportion of abnormal spirometry ranged from 32.3% to 53.8%. These proportions are similar to those cited in two case series of biopsy-documented constrictive bronchiolitis, which were 50% in the case of U.S. soldiers in Iraq and Afghanistan [King et al. 2011] and Iranians following sulfur mustard exposure, in which the pathology included proliferative bronchiolitis [Ghanei et al. 2008]. In the three large microwave popcorn plants, the proportion of mixed restrictive and obstructive spirometry in those with abnormal spirometry was similar to the proportion with pure obstructive and pure restrictive abnormalities. In the consecutive clinical case series [Markopoulou et al. 2002], the much lower proportion of restrictive abnormalities may be explained by the prevailing understanding a decade ago that obliterative bronchiolitis is an obstructive disease.

3.2.3 NIOSH Findings of Restrictive Spirometry at Flavoring Manufacturing Plants

As in the microwave popcorn investigations, flavoring manufacturing workforces with cases consistent with obliterative bronchiolitis have also had employees with restrictive spirometry, with proportions of restriction among those with abnormal spirometry ranging from 28.6% to 88.2% (Table 3-2).

NIOSH found an unusually high prevalence of a restrictive spirometric pattern among production employees at a flavoring manufacturing plant (Facility I) in Indiana [NIOSH 2011]. Among the 106 employees 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 employees 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 employees with serial abnormalities, 39 (37%) employees had abnormal findings. In comparison to the U.S. general population, the employee prevalence of restrictive spirometric abnormalities was 3.8 times higher than expected, after adjustment for race, ethnicity, sex, age, smoking status, and body mass index. NIOSH later detected an error in abstraction of smoking information from company spirometry reports and corrected this comparison to 3.7 [Kreiss 2014]. NIOSH also found evidence of rapid lung function decline in this workforce (section 3.3) with a 7.0-fold risk of excessive decline in the subgroup of production employees with higher potential for flavorings exposure (later corrected to 5.8) [Kreiss 2014]. Average declines in percent predicted FEV_1 and FVC for the employees with four annual measurements were in a pattern consistent with the evolution of restrictive lung disease. As in other flavoring plants, chemical exposures were diverse, although diacetyl was used nearly daily. Personal samples of diacetyl obtained by the company using NIOSH Method 2557 (uncorrected 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 correction) ranged to 1.9 ppm for personal and 2.9 ppm for area samples.

A company-sponsored re-analysis of Facility I spirometry data reported finding that no flavoring compounds, including diacetyl, had produced an increased risk of abnormal spirometric findings or longitudinal changes in spirometry [Ronk et al. 2013]. The study confirmed an excess risk of abnormal restrictive spirometry reported by NIOSH investigators with a similar prevalence ratio of 3.3 (95% CI 2.2–4.6) in comparison to the general population reflected in NHANES III. The authors offered the inadequacy of the NHANES III study population as a comparison group, despite adjusting for age, sex, and body mass index, because the national data were largely drawn from urban centers, and the authors alleged that the flavoring employees in a large city in Indiana were largely agrarian. As an alternative comparison group, the authors described the employee group with lower potential for flavoring exposure as an internal control group with no or minimal exposure, also referring to them as an administrative group. However, all employees in the medical surveillance program were in production areas, and company data documented measurable diacetyl in all production areas, including worrisome measurements in packaging which was classified in the NIOSH health hazard evaluation as having lower potential for exposure. Thus, the similar distribution of abnormal restrictive spirometry across the production workforce, without regard to higher and lower potential for flavoring exposure, remained unexplained and cannot be attributed to misclassification of lung disease by spirometry, variable quality spirometry, or body habitus, also mentioned by the authors. The most likely explanation for the 3.3–3.7 increased odds for restrictive disease in the Facility I workforce is that risk for work-related abnormality existed across both groups of production area employees in comparison to the national predicted estimate.

The Ronk et al. [2013] study conclusion that none of the flavoring compounds caused work-related spirometric abnormalities hinges on absence of association of pulmonary function abnormalities or decrements in employees with tenure in higher potential for flavoring exposure areas. The authors explain the difference in findings between their "negative" study and the NIOSH findings of work-related spirometric abnormalities by a NIOSH methodologic flaw in not taking account of correlated measures of serial lung functions. However, the authors misrepresent NIOSH analyses in which the outcome variables were the slopes of spirometric changes, expressed as mL/year, based on linear regression as a smoothing function. NIOSH also used categorical outcomes of excessive spirometric decline. Neither of these NIOSH outcomes reflected correlated serial data. In addressing serial (correlated) spirometry measures, Ronk et al. [2013] used generalized estimating equation modeling, which is a reasonable approach. However, the authors chose an exchangeable correlation structure, which assumes that the variation between any two measures is equal; this assumption would not appear appropriate for pulmonary function test measures at varying intervals. Measures taken at a 6-month interval would likely be more correlated than measures at several-year intervals, as occurred in the Facility I spirometry data set. The generalized estimating equation models assume that cumulative tenure is linearly related to the change in spirometry measures, which may not be the case in a short-latency health effect as has occurred in flavoring-exposed employees. The Ronk et al. [2013] paper omits report of average changes in FEV_1 and FVC per year in their model without workplace covariates, which might have indicated unusually high average decrements per year. NIOSH had found that the average FVC decline in the employee population was 108 mL/year, about 3.5-fold the expected decline of approximately 30 mL/year.

Ronk et al. [2013] separately modeled tenure in work areas with higher potential for exposure and tenure in liquid compounding with the apparent assumption that the remainder of the plant population had zero tenure (exposure), which is simply false. In particular, the liquid compounding tenure model ignores tenure in other higher potential for exposure jobs, which would clearly result in no associations with their work parameters. In contrast, the simpler NIOSH analyses of decline in lung function by areas with higher and lower potential for flavoring exposure demonstrated that both average declines and excessive decline differed between the two groups of production employees in statistically significant ways. These simple methods were not affected by correlated measurements.

A subsequent Indiana Occupational Safety and Health Administration (IOSHA) compliance investigation of Facility I reported hydrogen sulfide exposures above the NIOSH level immediately dangerous to life and health of 100 ppm [IOSHA 2012]. Hydrogen sulfide can result in obliterative bronchiolitis. IOSHA measured high concentrations of diacetyl (well above the proposed recommended exposure limit) in the packaging area that NIOSH had classified as lower potential for exposure. Thus, the diversity of exposures encountered by employees in this flavoring facility precluded identifying a specific cause(s) of the excess lung disease. However, the burden of likely occupational disease, reflected in the excess of restrictive spirometry and excessive annualized decline in spirometry, requires control of flavoring vapors, flavoring-related particulates, and hydrogen sulfide.

NIOSH found a high prevalence of a restrictive pattern on spirometry among employees at a plant (Facility E) where production employees combined liquid and powdered flavorings with flour, sugar, salt and other solid ingredients to produce baking mixes [NIOSH 2009b]. Of 41 employees, 23 (including 18 of 27 production employees) participated in a NIOSH medical survey that included spirometry testing. Of 22 employees 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 method under development, in-tube derivatization with 1,2-phenylenediamine (section 2.2.5 above), 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 [Day et al. 2011].

In 2008 NIOSH conducted an HHE of three cafeterias located at three different office buildings in New York City (Facilities F) [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 employees completed the questionnaire; 104 of 111 employees who underwent spirometry testing had a valid test). Five employees (5%) had airways obstruction, and two of these five employees had fixed obstruction. Both employees with fixed obstruction had started work at their current facility after butter-flavored cooking products were no longer in use. All five employees with obstruction denied having ever worked as professional cooks. Fifteen employees (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]. Five of the 15 had body mass indices over 30. Only three of the 15 reported cooking experience, and 13 reported cleaning experience. Compared to employees who did not cook at work, employees 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. Employees 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 employees who did not clean at work. Employees 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.

In these field investigations in microwave popcorn production, flavoring production, and food preparation, clinical evaluations of employees with spirometric restriction are unavailable, with the exception of the two employees at Facility G [Akpinar-Elci et al. 2004; Kreiss 2012]. One reason for the absence of pathophysiologic data is the previous focus of NIOSH investigators and clinicians on obstructive lung disease. Although the evolving literature now documents that obliterative bronchiolitis can manifest with normal or restrictive spirometry as well, NIOSH did not examine evidence for work-relatedness of restrictive disease and FEV_1 decline until reporting the findings in 2011 from medical surveillance data for flavoring Facility I [Kreiss 2014; NIOSH 2011]. A published case report exists of BOOP in an employee with exposure to spices and flavorings in making snack foods [Alleman and Darcey 2002] which has resulted in permanent impairment 10 years later [NIOSH unpublished data]; the role of flavorings in this case with restriction remains unclear. Because obstructive abnormalities are insensitive for pathologic obliterative bronchiolitis, future work should evaluate dyspnea and any spirometric abnormalities.

3.3 Rapid Lung Function Decline

Indirect and direct evidence shows that employees exposed to flavoring-related compounds can experience excessive lung function decline, whether within the normal range of spirometry or in those with either restrictive or obstructive

spirometric abnormalities. Indirect evidence comes from reviews of medical records and work histories of flavoring-exposed employees who developed obliterative bronchiolitis. In a case series summarizing the eight affected former employees and one additional current employee at the index microwave popcorn plant (Facility G), 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 manufactured flavors for the baking industry (Facility A), two flavoring production employees developed respiratory symptoms and severe fixed airways obstruction within 7 months of starting work at the plant [NIOSH 1986]. Although these employees 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 employee to lift 50- to 100-pound containers. It is unlikely that employees could have performed such tasks if their lung function was already severely compromised when they started work. Some affected employees stopped working when they could no longer do the job because of severe shortness of breath on exertion, while others were relocated to less strenuous jobs [NIOSH 1986, 2007a, 2008]. Severe airways obstruction as seen in obliterative bronchiolitis 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 1,000 people) [CDC 1996].

Direct evidence that employees exposed to flavoring-related compounds can experience rapid lung function decline comes from exposed employees 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 [Redlich et al. 2014]. Three of the affected former employees from the index microwave popcorn plant (Facility G) 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]. The investigators chose as the criterion for rapid decline a decrease in FEV_1 of 300 mL and/or 10% from an employee's initial (baseline) spirometry test to the employee's last spirometry test. This criterion was similar to a threshold developed based on a study of coal miners evaluated over time with spirometry of high technical quality 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 sensitive 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 technical 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 (Facility G) 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 employees had worked at some point in the mixing room, including one employee who experienced a 1,300-mL decline from the first test in November 2000 to the next test 5 months later; the next spirometry test 4 months after the second test showed an additional decline in FEV_1 of 600 mL, resulting in the employee leaving employment. This employee's FEV_1 continued to fall after leaving employment, with a total fall of 2,800 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 employees 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 employee had developed severe fixed airways obstruction [NIOSH 2008]. Another flavoring production employee at this plant had borderline airways obstruction on his first spirometry test, which is defined as a normal FEV_1 with a FEV_1/FVC ratio below the lower limit of normal. This employee 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 excessive lung function decline among flavoring production employees at a flavoring manufacturing company (Facility I) in Indiana [NIOSH 2011]. Diacetyl was used nearly daily in the plant and was measured in the air 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 employees. Interpretable spirometry results were available for 106 current and former production employees. NIOSH compared the results of each employee'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 employees 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 spirometry longitudinal data analysis (SPIROLA), a NIOSH freeware program that adjusts for data quality (within-person variation) and length of follow up [NIOSH 2010], NIOSH found that 19% (13) of employees 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. Employees currently working in areas with higher potential of flavorings exposure had 7.0-fold odds (later corrected to 5.8) [Kreiss 2014] of having excessive FEV_1 decline (95% CI 1.3–38.2, corrected to 1.2–28.8) in comparison to employees who were not currently working in areas with higher potential for exposure. The areas with higher potential for flavorings exposure included dry blend, extract and distillation, liquid compounding, process flavors, and spray dry areas. The

employees in these areas had 2.8 times greater average annual declines in FEV_1 than employees in other areas. The 18 production employees who had annual tests for 4 years (2006–2009) had average changes in their percent predicted FEV_1 and FVC measurements that declined in parallel with stable FEV_1/FVC ratios suggestive of an average tendency toward evolution of restrictive spirometry. Historical measurements of diacetyl and other flavoring compounds were insufficient to evaluate quantitative exposure-response relations. NIOSH also found a high prevalence (28%) of a restrictive pattern on spirometry tests in this workforce (section 3.2.3).

Company-sponsored re-analyses of the longitudinal spirometry data using generalized estimating equation models were interpreted as not showing any exposure-related declines in longitudinal spirometry measures [Ronk et al. 2013]. However, as noted earlier, the paper used an internal control group of production employees with diacetyl exposure as a control group, assumed zero tenure (reflecting zero exposure) for subgroups in the lower potential for exposure "control" group, and an exchangeable correlation structure for modeling that is not suitable for differing intervals of spirometric measures. See section 3.2.3 for further details.

The California Department of Public Health received serial spirometry test data for 416 flavor manufacturing employees administered from 2004 until early 2009, of whom 9.6% (40) had abnormal FEV_1 decline [Kreiss et al. 2012]. Abnormal FEV_1 decline rates (per person-month of follow up) were greater at companies using ≥ 800 lbs/year diacetyl than at companies using lesser amounts (7.3 versus 3.0 per 1,000 person-months, $P = 0.01$) and greater in companies previously shown to have four-person clusters of spirometric obstruction than at companies with no or only one employee with obstruction. Using only high quality serial spirometry data on a subset of 289 employees, 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 employee lost 23.9% (−980 mL) of his baseline FEV_1 over 25 months. Only five of the 21 employees had abnormal restrictive spirometry on one or more tests, three of whom developed restriction on their last test. The remaining 15 employees with excessive FEV_1 decline were within the normal range of FEV_1. 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 employees 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 examined rapid declines in flavoring-exposed or diacetyl-exposed employees. 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 contrast, 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 employees using a criterion of a greater than 320 mL or 8% decline over one year), adjusted for pack-years of smoking and body mass index [Lockey et al. 2009].

As indicated in the studies above, different approaches have been used by investigators over time to define excessive or rapid decline in FEV_1. These include percentage decline with various criteria, absolute decline with various criteria, normative population-based criteria for longitudinal limits of decline over various time intervals, and spirometry quality-adjusted

criteria, all of which are discussed in Chapter 9, section 9.5.

3.4 Asthma

At the index microwave popcorn plant and at one of the other five microwave popcorn plants that NIOSH evaluated (Facilities G and L), the prevalence of self-reported physician-diagnosed asthma was approximately two times higher than expected [NIOSH 2004b, 2006]. This suggests the possibility that some employees exposed to diacetyl and other flavoring compounds may be at increased risk for asthma (reversible airways obstruction) while others might be at risk for obliterative bronchiolitis (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 and that asthma may have been a misdiagnosis. Some employees 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 obliterative bronchiolitis [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. Many asthmatics react nonspecifically with bronchospasm to strong odors. Diacetyl has been reported to be a sensitizer in a rodent local lymph node assay, and other diketones, including 2,3-pentanedione, 2,3-hexanedione, 3,4-hexanedione and 2,3-heptanedione, have similar potency as sensitizers [Anderson et al. 2013]. Some aldehydes found in flavoring manufacturing plants are sensitizers. If sensitization to diacetyl or another chemical were to occur in a susceptible individual, that individual might develop allergic-type asthma, with diacetyl exposure triggering airways obstruction and respiratory symptoms. In the coffee manufacturing plant investigation (Facility R), evidence for occupational asthma among current and former employees consisted of sensitization to coffee and castor bean antigens known to be exposures in the industry, and exacerbation of asthma was reported in relation to roasting area smoke and dusts [Bailey et al. 2015].

NIOSH conducted an HHE at a small plant (Facility M) where employees 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 employees (lifelong nonsmokers) who had ever worked at the company developed respiratory disease while working there. One former employee had a mixed pattern of airways obstruction and restriction on spirometry; the airways obstruction was responsive to administered bronchodilator. This employee 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 employees who had symptoms of asthma, one had an FEV_1 that improved by 480 mL (11%) and an FVC by 510 mL (8%) within the normal ranges after bronchodilator administration. The other employee had abnormal airways resistance of 322% of predicted; 19% improvement of the mid-maximal forced expiratory flow after bronchodilator; and improvement in FEV_1 of 6% after bronchodilator. While employed at the plant, all three employees experienced worsening of their respiratory symptoms on the days they worked. HRCT scans of the chest showed findings suggesting possible bronchiolitis obliterans in the employee who died and in one of

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

3.6 Dermatologic Effects

Of the former employees who developed findings consistent with obliterative bronchiolitis while working at the index microwave popcorn plant (Facility G), one employee also developed a severe skin rash [Akpinar-Elci et al. 2004]. The employee 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 employee'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 (Facility N) NIOSH evaluated [NIOSH 2003a] to 36% among production employees at a flavoring plant (Facility B). Post-hire skin problems were reported by 60% of employees who primarily made liquid flavorings at this plant [NIOSH 2007a].

3.7 Discussion

Medical evaluations of employees 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 obliterative bronchiolitis. Some affected employees have experienced extremely rapid declines in lung function, with severe airways obstruction occurring within several months of the start of exposure to flavoring compounds [Akpinar-Elci et al. 2004; NIOSH 1986]. Whether restrictive lung disease is part of the spectrum of obliterative bronchiolitis in flavoring-exposed employees remains incompletely evaluated, although restrictive spirometry has been a common finding; in one plant, excessive FEV_1 declines in a restrictive pattern appear to be associated with potential for flavorings exposure. Employees as young as 22 years old have been affected by obstructive disease. Some affected employees have been placed on lung transplant waiting lists by their physicians because of the severity of their disease [Akpinar-Elci et al. 2004], and some flavoring-exposed employees have received lung transplants. 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 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].

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 employees developed progressive shortness of breath within months of starting work and then were found to have severe fixed airways obstruction [Kreiss et al. 2002; NIOSH 1986; van Rooy et al. 2007]. Additionally, NIOSH documented rapid falls in lung function in exposed employees with initially normal spirometry at three plants [NIOSH 2006, 2008, 2011]. Lockey et al. reported at the 2002 American Thoracic Society International Conference that five flavoring employees who developed moderate or severe fixed airways obstruction had normal spirometry at the start of employment [Lockey et al. 2009]. California public health surveillance showed that excessive FEV_1 decline occurred in employees in flavor manufacturing plants that participated in a preventive program attempting to lower flavoring exposures [Kreiss et al. 2012].

Temporality requires the exposure to precede disease development, and the inverse is that new disease cases should decline in a population with *cessation of exposure*, an evaluation by intervention or "experiment". Follow-up medical and environmental surveys at the index microwave popcorn plant (Facility G) revealed evidence of decreased lung disease risk with control of exposures. In employees 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, employees 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 employees hired before exposures were controlled, and these findings did not change significantly over time [Kanwal et al. 2011; NIOSH 2006]. Additionally, among 27 employees 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 employee 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 (Facility G), the prevalence of airways obstruction among nonsmoking current employees 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 employee surveillance data, the prevalence of severe airways obstruction was approximately three times higher than expected among all employees compared to national data. The prevalence in employees less than 40 years old was 15 times higher than expected [Kim et al. 2010].

The criterion of *replication of findings* (and *strength of the association*) between diacetyl exposure and development of severe occupational lung disease is apparent in the number of plants where employees have been affected and the number of production employees in these plants. The six microwave popcorn plants NIOSH evaluated represent a large segment of the microwave popcorn industry in the United States. Employees who developed findings consistent with obliterative bronchiolitis 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 1,000, 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 employees developed findings consistent with obliterative bronchiolitis at three flavoring plants (Facilities A, B, and C) where NIOSH conducted medical surveys. There were approximately 30 production employees across these three plants at the time of the NIOSH HHEs [NIOSH 1986, 2007a, 2008].

Consistency is also supported by the occurrence of lung disease consistent with obliterative bronchiolitis in diacetyl-exposed employees in at least eight flavoring manufacturing plants, a diacetyl production plant, a cookie manufacturing plant, and a coffee production plant [Akpinar-Elci et al. 2004; CDC 2007; Kanwal et al. 2006; Kim et al. 2010; NIOSH 1986, 2007a, 2008; 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].

Additional criteria to support a causal link between diacetyl exposure and severe lung

disease include *biologic plausibility, dose-response relationship,* and *consideration of alternate explanations. Biologic plausibility* is supported by experimental studies of diacetyl toxicity summarized in Chapter 4.

NIOSH found evidence of a *dose-response relationship* (i.e., worse lung disease or more employees affected with higher diacetyl exposure) in analyses of medical survey data from the index microwave popcorn plant (Facility G) 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 employees 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 employee surveillance data. An analysis of obstruction by amount of plant diacetyl use showed that there were 16 employees with obstruction in four companies that used more than 800 pounds of diacetyl annually compared to two employees 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 employees with severe fixed airways obstruction and other findings of obliterative bronchiolitis, 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 employees for the following reasons:

(1) Most affected employees 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 employees were away from the workplace as would be expected in employees with occupational asthma (either new onset asthma or exacerbation of pre-existing asthma).

(3) Employees' illnesses did not improve when they took medications for asthma such as bronchodilators and corticosteroids.

(4) Most employees 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 employees who developed severe lung disease were smokers, the natural history of smoking-related disease and the results of medical evaluations of affected employees make it unlikely that the cases of severe fixed airways obstruction among diacetyl-exposed employees 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 typically 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 does not occur before 55–60 years of age [Wise 2008]. Several diacetyl-exposed employees developed severe fixed airways obstruction while still in their 20s and 30s. Any smoking history among these affected employees (as well as in affected employees younger than 50) would not explain their severe fixed airways obstruction. Additional evidence against smoking as a cause of severe lung disease in these employees is the fact that most employees' DL_{CO} measurements were normal. In airways obstruction due to smoking-related emphysema, DL_{CO} is reduced.

Obliterative bronchiolitis is known to occur as a result of a variety of infections, exposures, or nonpulmonary diseases. Examples include 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, or in organ transplant recipients. The diacetyl-exposed employees who developed severe fixed airways obstruction did not have histories or medical evaluation findings to suggest that they had developed obliterative bronchiolitis from another exposure or medical condition. Airways obstruction can also occur due to diseases that affect other airways besides the bronchioles such as bronchiectasis or 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 employees who developed severe fixed airways obstruction.

Investigations of severe lung disease consistent with obliterative bronchiolitis among diacetyl-exposed employees 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 employees did not identify alternative explanations for their illness besides their workplace exposure to diacetyl and other flavoring compounds. Accordingly, the criteria for interpreting epidemiologic associations as causal have all been met by the body of investigation presented in this criteria document for a recommended standard.

References

Akpinar-Elci M, Travis WD, Lynch DA, Kreiss K [2004]. Bronchiolitis obliterans syndrome in popcorn production plant workers. Eur Respir J *24*(2):298–302.

Alleman T, Darcey DJ [2002]. Case report: bronchiolitis obliterans organizing pneumonia in a spice process technician. J Occup Environ Med *44*(3):215–216.

Anderson SE, Franko J, Wells JR, Lukomska E, Meade BJ [2013]. Evaluation of the hypersensitivity potential of alternative butter flavorings. Food Chem Toxicol *62*C:373–381.

ATS, ERS (American Thoracic Society, European Respiratory Society) [2002]. American Thoracic Society/European Respiratory Society international multidisciplinary consensus classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med *165*(2):277–304.

Bailey RL, Cox-Ganser JM, Duling MG, LeBouf RF, Martin SB, Bledsoe TA, Green BJ, Kreiss K [2015]. Respiratory morbidity in a coffee processing workplace with sentinel obliterative bronchiolitis cases. Am J Ind Med *58*(12):1235–1245.

Boros PW, Franczuk M, Wesolowski S [2004]. Value of spirometry in detecting volume restriction in interstitial lung disease patients. Spirometry in interstitial lung diseases. Respiration *71*(4):374–379.

Boylstein R [2012]. Identification of diacetyl substitutes at a microwave popcorn production plant. J Occup Environ Hyg *9*(2):D33–34.

California Department of Public Health [2007]. Food flavoring workers with bronchiolitis obliterans following exposure to diacetyl—California. http://www.cdph.ca.gov/programs/ohb/Documents/flavoringcases.pdf. Date accessed: July 2016.

Cavalcanti ZD, Albuquerque Filho AP, Pereira CA, Coletta EN [2012]. Bronchiolitis associated with exposure to artificial butter flavoring in workers at a cookie factory in Brazil. Jornal Brasileiro De Pneumologia *38*(3):395–399.

CDC (Centers for Disease Control and Prevention) [1996]. Third national health and nutrition examination survey, 1988–1994, NHANES III examination data file. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. (Public use data file documentation No. 76300).

CDC [2002]. Fixed obstructive lung disease in workers at a microwave popcorn factory—Missouri, 2000–2002. MMWR *51*(16):345–347.

CDC [2007]. Fixed obstructive lung disease among workers in the flavor-manufacturing industry—California, 2004–2007. MMWR *56*(16):389–393.

CDC [2013]. Obliterative bronchiolitis in workers in a coffee-processing facility—Texas, 2008-2012. MMWR *62*(16):305–307.

Cox-Ganser J, Ganser G, Saito R, Hobbs G, Boylstein R, Hendricks W, Simmons M, Eide M, Kullman G, Piacitelli C [2011]. Correcting diacetyl concentrations from air samples collected with NIOSH method 2557. J Occup Environ Hyg *8*(2):59–70.

Cummings KJ, Boylstein RJ, Stanton ML, Piacitelli CA, Edwards NT, LeBouf RF, Kreiss K [2014]. Respiratory symptoms and lung function abnormalities related to work at a flavouring manufacturing facility. Occupational and Environmental Medicine *71*(8):549–554.

Day G, LeBouf R, Grote A, Pendergrass S, Cummings K, Kreiss K, Kullman G [2011]. Identification and measurement of diacetyl substitutes in dry bakery mix production. J Occup Environ Hyg *8*(2):93–103.

Estenne M, Maurer JR, Boehler A, Egan JJ, Frost A, Hertz M, Mallory GB, Snell GI, Yousem S [2002]. Bronchiolitis obliterans syndrome 2001: An update of the diagnostic criteria. J Heart Lung Transplant *21*(3):297–310.

Ghanei M, Fathi H, Mohammad MM, Aslani J, Nematizadeh F [2004a]. Long-term respiratory disorders of claimers with subclinical exposure to chemical warfare agents. Inhal Toxicol *16*(8):491–495.

Ghanei M, Mokhtari M, Mohammad MM, Aslani J [2004b]. Bronchiolitis obliterans following exposure to

sulfur mustard: chest high resolution computed tomography. Eur J Radiol *52*(2):164–169.

Ghanei M, Tazelaar HD, Chilosi M, Harandi AA, Peyman M, Akbari HM, Shamsaei H, Bahadori M, Aslani J, Mohammadi A [2008]. An international collaborative pathologic study of surgical lung biopsies from mustard gas-exposed patients. Respir Med *102*(6):825–830.

Gordis L [1996]. Epidemiology. Philadelphia, PA: W. B. Saunders Company, pp. 176–177.

Halldin CN, Suarthana E, Fedan KB, Lo YC, Turabelidze G, Kreiss K [2013]. Increased respiratory disease mortality at a microwave popcorn production facility with worker risk of bronchiolitis obliterans. Plos One *8*(2):e57935.

Hankinson JL, Odencrantz JR, Fedan KB [1999]. Spirometric reference values from a sample of the general U.S. population. Am J Respir Crit Care Med *159*(1):179–187.

Hill AB [1965]. The environment and disease: association or causation? Proc R Soc Med *58*:295–300.

IOSHA [2012]. Safety order and notification of penalty. Inspection number 315051318, issued 06/18/2012; Final order by IOSHA Board of Safety Review, Case Docket No. 12-006 and 12-015, issued 01/04/2013. http://www.in.gov/dol/files/Sensient_No._315051318.pdf. Date accessed: July 2016.

Kanwal R [2008]. Bronchiolitis obliterans in workers exposed to flavoring chemicals. Curr Opin Pulm Med *14*(2):141–146.

Kanwal R, Kullman G, Fedan K, Kreiss K [2011]. Occupational lung disease risk and exposures to butter flavoring chemicals after implementation of controls at a microwave popcorn plant. Public Health Rep 126:480–494.

Kanwal R, Kullman G, Piacitelli C, Boylstein R, Sahakian N, Martin S, Fedan K, Kreiss K [2006]. Evaluation of flavorings-related lung disease risk at six microwave popcorn plants. J Occup Environ Med *48*(2):149–157.

Kim T, Materna B, Prudhomme J, Fedan K, Enright PL, Sahakian N, Windham G, Kreiss K [2010]. Industry-wide medical surveillance of California flavoring manufacturing workers: cross-sectional results. Am J Ind Med *53*(9):857–865.

King MS, Eisenberg R, Newman JH, Tolle JJ, Harrell FE, Jr., Nian H, Ninan M, Lambright ES, Sheller JR, Johnson JE, Miller RF [2011]. Constrictive bronchiolitis in soldiers returning from Iraq and Afghanistan. N Engl J Med *365*(3):222–230.

King T [2003]. Bronchiolitis. In: Schwartz MI, King T, eds. Interstitial lung disease. 4th ed. Hamilton, Ontario: BC Decker, Inc., pp. 787–824.

King T, Kinder BW [2008]. Bronchiolitis. In: Fishman AP, ed. Fishman's pulmonary diseases and disorders. 4th ed. New York: McGraw-Hill, pp. 887–911.

Kreiss K [2007]. Flavoring-related bronchiolitis obliterans. Curr Opin Allergy Clin Immunol *7*(2):162–167.

Kreiss K [2012]. Respiratory disease among flavoring-exposed workers in food and flavoring manufacture. Clin Pulm Med *19*(4):165–173.

Kreiss K [2014]. Work-related spirometric restriction in flavoring manufacturing workers. Am J Ind Med *57*(2):129–137.

Kreiss K, Fedan KB, Nasrullah M, Kim TJ, Materna BL, Prudhomme JC, Enright PL [2012]. Longitudinal lung function declines among California flavoring manufacturing workers. Am J Ind Med *55*(8):657–668.

Kreiss K, Gomaa A, Kullman G, Fedan K, Simoes EJ, Enright PL [2002]. Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. N Engl J Med *347*(5):330–338.

Lockey JE, Hilbert TJ, Levin LP, Ryan PH, White KL, Borton EK, Rice CH, McKay RT, LeMasters GK [2009]. Airway obstruction related to diacetyl exposure at microwave popcorn production facilities. Eur Respir J *34*(1):63–71.

Markopoulou KD, Cool CD, Elliot TL, Lync DA, Newell JD, Jr., Hale VA, Brown KK, Schwarz MI, Tuder RM [2002]. Obliterative bronchiolitis: varying presentations and clinicopathological correlation. Eur Respir J *19*(1):20–30.

NIOSH [1986]. Hazard evaluation and technical assistance report: International Bakers Services, Inc, South Bend, IN. By McConnell R, Hartle R. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 1985–0171–1710.

NIOSH [2003a]. Hazard evaluation and technical assistance report: Agrilink Foods Popcorn Plant, Ridgway, IL. By Sahakian N, Choe K, Boylstein R, Schleiff P. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2002–0408–2915.

NIOSH [2003b]. Hazard evaluation and technical assistance report: B.K. Heuermann Popcorn, Inc, Phillips, NE. By Kanwal R, Martin S. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2001–0517.

NIOSH [2003c]. Hazard evaluation and technical assistance report: Nebraska Popcorn, Clearwater, NE. By Kanwal R. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2002–0089.

NIOSH [2004a]. Hazard evaluation and technical assistance report: American Pop Corn Company, Sioux City, IA. By Kanwal R, Boylstein R, Piacitelli C. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2001–0474–2943.

NIOSH [2004b]. Hazard evaluation and technical assistance report: ConAgra Snack Foods, Marion, OH. By Kanwal R, Kullman G. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2003–0112–2949.

NIOSH [2006]. Hazard evaluation and technical assistance report: Gilster-Mary Lee Corporation, Jasper, MO. By Kanwal R, Kullman G, Fedan K, Kreiss K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2000–0401–2991.

NIOSH [2007a]. Hazard evaluation and technical assistance report: Carmi Flavor and Fragrance Company, Inc., Commerce, CA. By Kanwal R, Kullman G. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2006–0303–3043.

NIOSH [2007b]. Hazard evaluation and technical assistance report: Yatsko's Popcorn, Sand Coulee, MT. By Kullman G, Sahakian N. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2006–0195–3044.

NIOSH [2008]. Hazard evaluation and technical assistance report: Gold Coast Ingredients, Inc., Commerce, CA. By Bailey R, McKernan L, Dunn K, Sahakian N, Kreiss K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2007–0033–3074.

NIOSH [2009a]. Flavorings-related lung disease. http://www.cdc.gov/niosh/topics/flavorings. Date accessed: July 2016.

NIOSH [2009b]. Hazard evaluation and technical assistance report. Report on an investigation of buttermilk flavoring exposures and respiratory health at a bakery mix production facility, General Mills, Los Angeles, CA. By Day G, Cummings K, Kullman G. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2008–0230–3096.

NIOSH [2009c]. Hazard evaluation and technical assistance report. Respiratory symptoms in workers at three commercial kitchens, Aramark, New York, NY. By Gaughan D, Boylstein R, Iossifova Y, Piacitelli C, Bailey R, Day G. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2008–0125–0126–0127–3093.

NIOSH [2009d]. Hazard evaluation and technical assistance report: Chr. Hansen, Inc., New Berlin, WI. By Sahakian N, Kullman G, Dunn K, Kanwal R. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2007–0327–3083.

NIOSH [2010]. Spirometry in the occupational setting: Spirometry Longitudinal Data Analysis (SPIROLA) software. Atlanta, GA: Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Version 3.0, updated November 2010. http://

www.cdc.gov/niosh/topics/spirometry/spirola.html. Date accessed: July 2016.

NIOSH [2011]. Hazard evaluation and technical assistance report: Lung function (spirometry) testing in employees at a flavorings manufacturing plant—Indiana. By Kreiss K, Piacitelli C, Cox-Ganser J. Morgantown, WV: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2008–0155–3131.

NIOSH [2013]. Hazard evaluation and technical assistance report: An investigation of respiratory health at a flavoring manufacturing facility. By Cummings K, Boylstein R, Stanton M, Piacitelli C. Morgantown, WV: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA No. 2012-0012-3192.

Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF, Wanger J [2005]. Interpretative strategies for lung function tests. Eur Respir J *26*(5):948–968.

Redlich CA, Tarlo SM, Hankinson JL, Townsend MC, Eschenbacher WL, Von Essen SG, Sigsgaard T, Weissman DN [2014]. Official American Thoracic Society technical standards: spirometry in the occupational setting. Am J Respir Crit Care Med *189*(8):983–993.

Ronk CJ, Hollins DM, Jacobsen MJ, Galbraith DA, Paustenbach DJ [2013]. Evaluation of pulmonary function within a cohort of flavorings workers. Inhalation Toxicology *25*(2):107–117.

Rowell M, Kehe K, Balszuweit F, Thiermann H [2009]. The chronic effects of sulfur mustard exposure. Toxicology *263*(1):9–11.

Ryu JH, Scanlon PD [2001]. Obstructive lung diseases: COPD, asthma, and many imitators. Mayo Clin Proc *76*(11):1144–1153.

Sahakian N, Kullman G, Lynch D, Kreiss K [2008]. Asthma arising in flavoring-exposed food production workers. Int J Occup Med Environ Health *21*(2):173–177.

Schachter EN [2002]. Popcorn worker's lung. N Engl J Med *347*(5):360–361.

Schlesinger C, Meyer CA, Veeraraghavan S, Koss MN [1998]. Constrictive (obliterative) bronchiolitis: diagnosis, etiology, and a critical review of the literature. Ann Diagn Pathol *2*(5):321–334.

van Rooy FG, Rooyackers JM, Prokop M, Houba R, Smit LA, Heederik DJ [2007]. Bronchiolitis obliterans syndrome in chemical workers producing diacetyl for food flavorings. Am J Respir Crit Care Med *176*(5):498–504.

van Rooy FG, Smit LA, Houba R, Zaat VA, Rooyackers JM, Heederik DJ [2009]. A cross-sectional study of lung function and respiratory symptoms among chemical workers producing diacetyl for food flavourings. Occup Environ Med *66*(2):105–110.

Visscher DW, Myers JL [2006]. Bronchiolitis: the pathologist's perspective. Proc Am Thorac Soc 3(1):41–47.

Wang ML, Petsonk EL [2004]. Repeated measures of FEV_1 over six to twelve months: what change is abnormal? J Occup Environ Med *46*(6):591–595.

White KL, Heikkila K, Williams R, Levin L, Lockey JE, Rice C [2010]. Diacetyl exposures at four microwave popcorn plants. J Occup Environ Hyg *7*(4):185–193.

Wise RA [2008]. Chronic obstructive pulmonary disease: clinical course and management. In: Fishman A, ed. Fishman's pulmonary diseases and disorders, 4th ed. New York: McGraw–Hill, pp. 729–746.

4 Toxicology of Diacetyl and 2,3-Pentanedione

4.1 Chemistry and Metabolism

Chemical and physical properties of diacetyl and 2,3-pentanedione are presented in section 1.4. As mentioned there, diacetyl is an α-dicarbonyl. Endogenous α-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 α-dicarbonyl compounds, diacetyl is reactive, with a tendency to cause protein cross-links [Miller and Gerrard 2005]. The reactivity of the α-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 α-dicarbonyl compounds can inactivate proteins, principally through reactions with the amino acid, arginine [Epperly and Dekker 1989; Mathews et al. 2010; Saraiva et al. 2006]. The related α-dicarbonyl flavoring, 2,3-pentanedione, has been reported to be even more reactive with arginine groups than diacetyl [Epperly and Dekker 1989]. Recently, in vitro studies indicate that diacetyl can cause β-amyloid aggregates and covalently modify β-amyloid at 5th arginine (Arg^5), although the in vivo relevance of this finding remains to be investigated [More et al. 2012b]. Thus, existing studies indicate that diacetyl is a reactive compound that can modify proteins and suggest that diacetyl-associated protein modification may occur in vivo.

4.1.1 Diacetyl and 2,3-Pentanedione in Food

Diacetyl and 2,3-pentanedione have a long history as components of food, suggesting that exposures can occur in diverse workplaces. They occur as natural products in many foods [Jiang et al. 2013; Majcher and Jelen 2005; Majcher et al. 2013; Rincon-Delgadillo et al. 2012; Santos et al. 2013]. Diacetyl imparts the flavor and aroma of butter to many common foods and drinks including butter, cheese, yogurt, beer, and wine [Jang et al. 2013; Rincon-Delgadillo et al. 2012]. Diacetyl in food plays an important role in food preference [Liggett et al. 2008]. While less extensively studied in foods than diacetyl, 2,3-pentanedione is a common flavoring in margarine and vegetable spreads [Rincon-Delgadillo et al. 2012]. Roasted coffee also contains appreciable amounts of diacetyl [CDC 2013; Daglia et al. 2007a; Daglia et al. 2007b]. Because diacetyl is not a component of green coffee beans, it appears to be a product of the roasting process [Daglia et al. 2007a].

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 some starter distillate samples include diacetyl and acetic acid [FASEB 1980; Rincon-Delgadillo et al. 2012]. A recent study demonstrated that diacetyl remains a frequent component of commercial starter distillate samples, and diacetyl concentrations exceeded 20 mg/g in one sample [Rincon-Delgadillo et al. 2012]. Starter distillate can also contain 2,3-pentanedione as well as butyric acid, which inhibits the metabolism of diacetyl and 2,3-pentanedione [Morris and Hubbs 2009; Nakagawa et al. 2002a; Rincon-Delgadillo et al. 2012]. Thus, diacetyl and 2,3-penanedione can occur naturally in food, may be added to food as flavorings, may be produced during the roasting process, and can be anticipated components when starter distillate is added as a flavoring.

4.1.2 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), the enzyme is also known as diacetyl reductase, with either nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (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 [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 that is comprised of four subunits, each 244 amino acids 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].

Table 4-1. Experimental respiratory toxicology studies

Reference	Test subject	Exposure	Effects of exposure
Fedan et al. [2006]	In vitro preparations of guinea pig trachea	Diacetyl 1 mM 3 mM 10 mM 30 mM	Direct effect of diacetyl: Very weak tracheal contractions with threshold at 1 mM, relaxation at exposures above 3 mM. Diacetyl effect on response to intraluminal (mucosal) methacholine: 4-hour perfusion with 3 mM diacetyl increased methacholine reactivity 10 times; 10 mM diacetyl completely inhibited the methacholine response. Depolarization of transepithelial potential difference at 3 and 10 mM. Decrease in transepithelial potential difference at 10 mM.
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 employee is predicted to be more than 40 times the dose to the bronchiole of a rat. 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.
[Goravanahally et al. 2014]	Male Sprague-Daley Rats	6-hr diacetyl inhalation with sacrifice 18–20 hr post-exposure 0 ppm 25 ppm 249 ppm 346 ppm	Diacetyl increased substance P positive neurons in the jugular ganglia in a dose-dependent manner. PGP9.5 nerve fiber density was decreased in foci with denuded tracheal epithelium after inhaling 249 or 346 ppm diacetyl. Substance P-positive nerve fiber density was increased in foci of intact epithelium adjacent to denuded epithelium.
Hubbs et al. [2002]	Male Sprague-Dawley rats	Control (n=16) 0 ppm Diacetyl exposures were to vapors of a complex mixture of diacetyl-containing butter flavoring Exposure for 6 hr: Low constant exposure (n=6) 203 ppm of the diacetyl component	Airway epithelial damage with necrosis and inflammation in nose

See footnotes at end of table.

(Continued)

Table 4-1 (Continued). Experimental respiratory toxicology studies

Reference	Test subject	Exposure	Effects of exposure
		Middle constant exposure (n=3) 285 ppm of the diacetyl component	Airway epithelial damage with necrosis and inflammation in nose and lungs (bronchi)
		High constant exposure (n=3) 352 ppm of the diacetyl component	Airway epithelial damage with necrosis and inflammation in nose and lungs (bronchi)
		High pulsed exposure (n=3) 371 ppm (range 72–940) of the diacetyl component	Airway epithelial damage with necrosis and inflammation in nose and lungs (bronchi and bronchioles)
Hubbs et al. [2008]	Male Sprague-Dawley rats	Control (n=18) 0 ppm Diacetyl inhalation for 6 hr:	
		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 min diacetyl inhalation pulses in 6-hr (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 diacetyl inhalation exposure for 6 hr 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

See footnotes at end of table.

(Continued)

Table 4-1 (Continued). Experimental respiratory toxicology studies

Reference	Test subject	Exposure	Effects of exposure
		Single ~15 min pulse exposure 1949 ppm (92.9 6 hr TWA) (n=6)	Airway epithelial damage with necrosis and/or inflammation in nose (3/6)
Hubbs et al. [2012]	Male Sprague-Dawley rats	6-hr inhalation exposures to air, 2,3-pentanedione (PD) or diacetyl; sacrifice 18–20 hr after exposure.	
		Six exposure groups: Air 111.7 ± 0.12 ppm PD 241.2 ± 0.15 ppm PD 318.4 ± 0.17 ppm PD 354.2 ± 0.20 ppm PD 240.3 ± 0.26 ppm diacetyl	Apoptosis and necrosis of respiratory and transitional epithelium of the nose in all 2,3-pentanedione- and diacetyl-exposed rats; necrosis of olfactory neuroepithelium and necrotizing tracheitis in diacetyl exposed rats and rats inhaling ≥ 241.2 ppm 2,3-pentanedione; necrotizing bronchitis in rats inhaling ≥ 318.4 ppm 2,3-pentanedione
		6-hr inhalation exposures to air or 319 (317.9 to 318.9) ppm PD. Air exposed rats sacrificed at 18 hr post-exposure; 2,3-pentanedione exposed rats were sacrificed at 3 different post-exposure time points: 0–2 hr post-exposure 12–14 hr post-exposure 18–20 hr post-exposure	In the proximal nose, severity of damage increased between the 0–2 hr post-exposure and 12–14 hr post-exposure, suggesting delayed toxicity; disorganization of the neuroepithelium with a loss of the organized expression of DCXR at the air/olfactory neuroepithelium interface; activation of caspase-3 in olfactory axons
Larsen et al. [2009]	Male BALB/cJ mice	Diacetyl inhalation exposures to 191, 334, 79, or 1154 ppm	Pulmonary irritation at 790 and 1154 ppm, decrease in respiratory rate, increase in "time of break"
		Challenge after acute exposure 555 ppm	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 diacetyl inhalation, whole body 6 hr/day, 5 days	
		0 ppm	
		200 ppm	Necrosis and inflammation in mucosa of the nose and larynx
		400 ppm	Necrosis and inflammation in the mucosa of the nose, larynx and bronchi

See footnotes at end of table.

(Continued)

Table 4-1 (Continued). Experimental respiratory toxicology studies

Reference	Test subject	Exposure	Effects of exposure
		Intermittent diacetyl inhalation, nose only	
		0, 100, 200, or 400 ppm 1 hr/day, 5 days/week, 2 weeks	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)
		0, 100, 200, or 400 ppm 1 hr/day, 5 days/week, 4 weeks	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).
		0 or 1200 ppm 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 6 hr/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).

See footnotes at end of table.

(Continued)

Table 4-1 (Continued). Experimental respiratory toxicology studies

Reference	Test subject	Exposure	Effects of exposure
		0, 25, 50, or 100 ppm 6 hr/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 of diacetyl 0, 100, 200, or 400 mg/kg, single dose 4 days post-aspiration	Foci of fibrohistiocytic proliferation with little or no inflammation present at the junction of the terminal bronchioles and alveolar ducts (400 mg/kg)
Morgan et al. [2012]	Male and female Wistar-Han rats and B6C3F1 mice	2,3-Pentanedione 0, 50, 100 or 200 ppm 6 hr/day, 5 days/week, Up to 12 exposures	In rats, 12-day 2,3-pentanedione exposure cause necrosis of respiratory epithelium and atrophy of olfactory neuroepithelium in the nose; intramural and intraluminal fibrosis of intrapulmonary airways. In rats, 5 or 10 days of inhaling 200 ppm 2,3-pentanedione increased bronchoalveolar lavage fluid concentrations of monocyte chemotactic protein-1, monocyte chemotactic protein-3, C-reactive protein, fibroblast growth factor-9, and fibrinogen. In mice, 12-day 2,3-pentanedione exposure caused necrosis of nasal turbinates, suppurative exudate and atrophy of olfactory neuroepithelium in the nose; inflammation in intrapulmonary airways with necrosis, ulceration and regeneration at 200 ppm exposure.
Morris and Hubbs [2009]	Male Sprague-Dawley rats	Diacetyl 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 humans. 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 humans. Butyric acid is shown to shift diacetyl absorption from the nose and trachea into deeper lung tissue.

See footnotes at end of table.

(Continued)

Table 4-1 (Continued). Experimental respiratory toxicology studies

Reference	Test subject	Exposure	Effects of exposure
Palmer et al. [2011]	Male Sprague-Dawley Rats	200 µl of a 188 mg/mL diacetyl solution by intratracheal instillation (125 mg/kg)	Initial airway epithelial injury was followed by the development of obliterative bronchiolitis
Zaccone et al. [2013]	In vitro preparation of rat trachea after exposure of animals	Diacetyl 6-hr inhalation exposures to 100, 200, 300, or 360 ppm (n=6–11)	Small increase in basal airway compliance at 360 ppm Increase in reactivity at 320 and 360 ppm
		2,3-Pentanedione 6-hr inhalation exposures to 120, 240, 320, or 360 ppm (n=5–8)	No effect on basal airway resistance or compliance Increase in reactivity at 320 and 360 ppm
	In vivo exposure of male Sprague-Dawley rats	Diacetyl 6-hr inhalation exposures to 100, 200, 300, or 360 ppm	Decrease in reactivity at 360 ppm
		2,3-Pentanedione 6-hr inhalation exposures to 120, 240, 320, or 360 ppm (n=4–10)	Decrease in reactivity at 240 and 320 ppm

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, b; 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, aldo-keto reductase 1C15 is a newly-described aldo-keto reductase expressed in rat lung that can metabolize α-diketones [Endo et al. 2007]. Recently, a low affinity, high capacity and a high affinity, low capacity pathway for diacetyl metabolism have 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 occupational exposures.

4.2 In Vivo and in Vitro Toxicology Studies

Diacetyl and 2,3-pentanedione may be consumed in food, the vapors may be inhaled, and direct skin contact is possible. In vivo studies have modeled these routes of human exposure.

Table 4-1 summarizes key respiratory toxicology findings for diacetyl and 2,3-pentanedione through 2013. 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].

4.2.1 In Vivo Toxicology of Orally Administered Diacetyl

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 Effects of Topically Applied Diacetyl and 2,3-Pentanedione in Vivo

Sensitization following topical application of diacetyl is predicted on the basis of results of a murine local lymph node assay [Anderson et al. 2013; Anderson et al. 2007; Roberts et al. 1999].

On the basis of the results of the local lymph node assay and immune cell phenotyping, it is suggested that diacetyl and 2,3-pentanedione function as T-cell mediated chemical sensitizers [Anderson et al. 2013]. Cutaneous sensitization by diacetyl and 2,3-pentanedione may be initiated through haptenation with proteins containing the amino acids lysine and arginine [Roberts et al. 1999]. Diacetyl is corrosive to the cornea of the eye using the Draise test [Sugai et al. 1990].

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, a 6-hour diacetyl inhalation exposure caused epithelial necrosis and inflammation in bronchi at concentrations of ≥ 294.6 ppm and caused epithelial necrosis and inflammation in the trachea and larynx at concentrations of ≥224 ppm [Hubbs et al. 2008]. The airway epithelial damage in rats inhaling 356 ppm was remarkable, with an average pathology score of 9.5 on a 10-point scale in the nasopharyngeal duct and larynx, while damage in the tracheal epithelium averaged 8.7 on a 10-point scale. 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]. The data from the National Toxicology Program 90-day inhalation study are available online and was used for the NIOSH animal-based risk assessment (see Chapter 6). Airway damage in mice one day after diacetyl inhalation was found to correlate with markers of increased protein turnover, implicating protein damage in the etiology of diacetyl-induced airway damage [Hubbs et al. 2016].

Eighteen hours after a 6-hour exposure to inhaled diacetyl (100, 200, 300, or 360 ppm), in anesthetized rats 360 ppm elevated slightly lung resistance and dynamic compliance [Zaccone et al. 2013]. 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. Eighteen hours after a 6-hour exposure to inhaled 2,3-pentanedione (120, 240, 300, or 360 ppm), in anesthetized rats basal lung resistance (R_L) and dynamic lung compliance (C_{dyn}) were not affected.

Following inhalation of 346 ppm diacetyl by rats, foci in the trachea that demonstrate epithelial denudation also appear to have loss of sensory nerves, as reflected in a decreased density of PGP9.5-immunoreactive nerve fibers. However, in the epithelium adjacent to denuded foci, increased numbers of nerve fibers contain immunoreactive substance P, a neuropeptide important in neurogenic airway inflammation. In addition, the number of substance P immunoreactive neurons increase in the ganglia supplying the trachea in a dose-dependent manner in exposed rats. These findings suggest that diacetyl-induced airway epithelial damage may be accompanied by changes in the sensory nerves [Goravanahally et al. 2014]. 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 only 1 hour/day at 100, 200, or 400 ppm diacetyl, 5 days per week, for 2 to 4 weeks rather than 6 hours/day for the same number of days eliminated epithelial necrosis in mice inhaling 200 ppm diacetyl and decreased the severity of epithelial necrosis in mice inhaling 400 ppm diacetyl. 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 1,200 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 obliterative bronchiolitis in rats after intratracheal instillation of diacetyl [Palmer et al. 2011]. In this model, diacetyl-induced obliterative bronchiolitis was associated with abnormal repair of the injured bronchiolar epithelium. The reports of the induction of obliterative bronchiolitis and obliterative bronchiolitis-like changes in the deep lung of laboratory animals following aspiration of diacetyl are important because no prior animal model of obliterative bronchiolitis existed, and it is a technique that bypasses the rodent nose, which CFD-PBK models have demonstrated to absorb more diacetyl than will be absorbed in the upper airways of employees (section 4.2.6). 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]. A study demonstrated bronchial fibrosis in rats inhaling 150 or 200 ppm diacetyl for 2 weeks [Morgan et al. 2016].

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 employees during acute diacetyl exposures at concentrations less than 20 ppm [Larsen et al. 2009]. As mentioned earlier, a recent study suggests that acute diacetyl inhalation exposures can actually increase the number of substance P-positive neurons in the jugular ganglia and the number of nerve fibers containing substance P in the epithelium adjacent to sites of epithelial damage, but decreases the sensory innervation at the actual sites of greatest epithelial damage [Goravanahally et al. 2014]. 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 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 2,500 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. Phagocytosis of olfactory nerve material and increases in Tnfα mRNA were recently demonstrated in the olfactory bulb of mice one day after a 6-hr diacetyl inhalation exposure. By immunofluorescence, the multifunctional scaffolding protein sequestosome-1 accumulated in the olfactory bulb of these mice and often congregated in the microglial cells that contained phagocytized olfactory neuronal material [Hubbs et al. 2016].

Although powdered butter flavoring can produce fewer vapors than liquid butter flavorings, the powders have a major respirable component [Boylstein et al. 2006; Rigler and Longo 2010]. If powdered butter flavorings are substituted for liquid butter flavorings, diacetyl and 2,3-pentanedione vapor concentrations may well be below exposure limits. In particular, encapsulated flavoring powders are designed to contain diacetyl or 2,3-pentanedione vapors. However, inhalable particulates can be deposited in the nose, the conducting airways, and deep lung. No peer reviewed studies have investigated the potential for encapsulated flavorings to release diacetyl and/or 2,3-pentanedione directly to the target cells lining airways. However, a recent study indicates that more than a quarter of particulates in flavoring powders are less than 10 μm in diameter. Therefore, powders have the potential to reach the intrapulmonary airways [Rigler and Longo 2010].

4.2.4 In Vitro Toxicology of Diacetyl and 2,3-Pentanedione

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]. Consistent with these findings, recent in vitro studies of direct interactions between diacetyl and single-stranded oligonucleotides under acellular conditions indicate that diacetyl can form adducts with 2-deoxyguanosine [More et al. 2012a]. 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]. This concentration has been shown to have pharmacological activity in airways and has been modeled to be achieved in the airway wall after inhalation (see below). 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 [Sumegi et al. 1982].

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 or 2,3-pentanedione applied to the mucosal surface in concentrations 1 mM and higher dissolved in a physiological salt solution elicited small contractions; in concentrations higher than 3 mM (i.e., 10 and 30 mM), contractions to diacetyl and 2,3-pentanedione were followed by relaxations. The relaxation responses were larger than the contractile responses. Exploring these phenomena further, adding the flavorings 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 is produced.

Investigation of inhaled diacetyl effects (60, 100, 200, 300, and 360 ppm) and inhaled 2,3-pentanedione effects (120, 240, 320, and 360 ppm) on reactivity of tracheas removed from exposed rats and studied in the isolated, perfused trachea model showed that reactivity to methacholine applied to the mucosal surface was increased slightly after inhalation of 300 and 360 ppm diacetyl and 320 and 360 ppm 2,3-pentanedione. Based on epithelial damage in airways after exposure to diacetyl, a larger

increase in airway reactivity had been anticipated [Zaccone et al. 2013].

Diacetyl inhalation elicits substantial histopathologic changes to airway epithelium, including denudation and necrosis (section 4.2.3). 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 [Zaccone et al. 2013].

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 CFD-PBK 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].

2,3-Pentanedione is not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA102, or TA104 [Aeschbacher et al. 1989; Marnett et al. 1985].

A recent study demonstrates that in vitro diacetyl exposure increases shedding of the epidermal growth factor ligand, amphiregulin. These findings are further supported by evidence that amphiregulin transcripts and protein are also increased in an in vivo model of obliterative bronchiolitis induced by repeated intratracheal instillation of diacetyl [Kelly et al. 2014]. Amphiregulin has previously been reported to play a role in pulmonary fibrosis, although the nature of that role is not fully understood [Zhou et al. 2012].

4.2.5 Toxicology of Inhaled Diacetyl Substitutes

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, and it was present along with diacetyl in many of the workplaces where obliterative bronchiolitis occurred in employees who make or use diacetyl [Kullman et al. 2005; van Rooy et al. 2007]. Acetoin is structurally very similar to diacetyl but an α-hydroxyketone in acetoin replaces the reactive α-diketone implicated in the toxicity of diacetyl and 2,3-pentanedione [Hubbs et al. 2012]. In a National Toxicology Program (NTP) 90-day study on acetoin, the chosen maximum exposure level (generally representing the maximum tolerated dose) was 800 ppm, whereas in the NTP 90-day diacetyl study the maximum exposure level was 100 ppm (Chapter 6). In 90-day inhalation exposures, diacetyl produced statistically significant respiratory tract lesions from exposures as low as 25 ppm, in both rats and mice (Chapter 6). Statistically significant histopathology changes in the 25 ppm diacetyl exposures were nasal respiratory epithelial metaplasia (in both male and female rats), nasal olfactory epithelial degeneration in female rats; nasal respiratory epithelial necrosis (in male and female mice) plus chronic inflammation and respiratory epithelial hyperplasia of the larynx in female mice (Chapter 6). In addition acetoin (150 ppm), after inhalation for 6 hours by rats, had no effect on reactivity to inhaled methacholine while inhaled acetic acid (27 ppm for 6 hours), another component of flavoring mixture, increased airway reactivity to methacholine [Zaccone et al. 2013]. Thus, current data, while limited, indicate that acetoin is considerably less hazardous than diacetyl.

2,3-Pentanedione is structurally very similar to diacetyl because it is a 5-carbon α-diketone, and diacetyl is a 4-carbon α-diketone. Eighteen hours after a 6-hour inhalation exposure to 2,3-pentanedione (120, 240, 320, and 360 ppm), R_L and C_{dyn} in anesthetized rats were unaffected [Zaccone et al. 2013]. Subsequently, airway reactivity to inhaled methacholine aerosol was decreased after inhalation of 120, 240, and 320 ppm 2,3-pentanedione. 2,3-Pentanedione affected methacholine reactivity more than diacetyl. 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 [Zaccone et al. 2013]. The magnitude of this effect surpassed that caused by diacetyl inhalation.

Morphologic data suggest that 2,3-pentanedione can cause airway epithelial damage similar to the damage caused by diacetyl [Hubbs et al. 2012; Morgan et al. 2012; Morgan et al. 2016]. Rats repeatedly inhaling 2,3-pentanedione at concentrations ≥ 150 ppm for up to 2 weeks develop fibrosis of intrapulmonary airways, a morphologic change similar to obliterative bronchiolitis in humans [Morgan et al. 2016]. Recently, more than 3500 genes were found to be upregulated in RNA isolated from the fibrotic bronchi of 2,3-pentanedione exposed rats [Morgan et al. 2015]. Some of the up-regulated genes were ones previously implicated in fibrosis, including transforming growth factor-β2, interleukin-1α, interleukin-18, interleukin-33, and fibronectin. In addition, at high exposure concentrations, messenger RNA changes were noted in the brain of rats after acute 2,3-pentanedione inhalation [Hubbs et al. 2012].

4.2.6 Diacetyl and 2,3-Pentanedione in Cigarette Smoke

A recent study suggests that mainstream tobacco smoke collected by a smoking machine

contained significant amounts of diacetyl that varied with the smoking parameters set by different organizations. The average for seven types of cigarettes ranged between 285 μg diacetyl/cigarette and 42.8 μg 2,3-pentanedione/cigarette for the International Organization for Standardization (ISO) parameters to 778 μg diacetyl/cigarette and 83.5 μg 2,3-pentanedione/cigarette by the Health Canada Intensive (HCI) parameters. In mainstream cigarette smoke, the calculated diacetyl concentration ranged from 250 ppm with ISO parameters to 361 ppm with HCI parameters, while the concentration of 2,3-pentanedione ranged from 32.2 ppm with ISO parameters to 50.1 ppm with HCI parameters [Pierce et al. 2014]. Unfortunately, the analytical technique used in the study (high performance liquid chromatography with ultraviolet detection analyses of 2,4-dintrophenylhydrazine derivatives) is not especially selective and would be prone to interferences in complex mixtures. Nevertheless, two other recent studies support the presence of significant diacetyl in mainstream cigarette smoke [Fujioka and Shibamoto 2006; Polzin et al. 2007]. Other recent studies have identified diacetyl and 2,3-pentanedione in electronic cigarette liquids and aerosols [Allen et al. 2016; Farsalinos et al. 2015].

In one study, after derivatization with 1,2-phenylenediamine, quantification by GC-NPD, and confirmation by GC-MS, 301–433 μg diacetyl/cigarette was measured in the total mainstream smoke withdrawn from each burning cigarette from 15 different commercial reference cigarettes [Fujioka and Shibamoto 2006]. However, that study did not use a smoking machine to simulate actual smoking behavior, so that the amount of diacetyl observed may not be representative of the amount produced under realistic smoking conditions. In another study, using GC/MS and a smoking machine with ISO parameters, a range of 12.7–145 μg diacetyl/cigarette was measured from 41 different types of cigarettes [Polzin et al. 2007]. Thus, several studies suggest that significant diacetyl is present in mainstream cigarette smoke, although the concentrations vary [Fujioka and Shibamoto 2006; Pierce et al. 2014; Polzin et al. 2007]. Finally, electronic cigarette liquids often contain diacetyl and 2,3-pentanedione [Allen et al. 2016; Farsalinos et al. 2015].

The Pierce et al. [2014] report is the first to calculate diacetyl concentrations in mainstream cigarette smoke in ppm. However, it should be noted that the calculated concentration in ppm such as the reported average concentration of 250 ppm diacetyl using the ISO parameters is actually not comparable to workplace exposures [Pierce et al. 2014]. Using ISO parameters an average of 0.3255 liters of mainstream cigarette smoke was inhaled, but this would not be the only air inhaled by a smoker. Roughly 6.75 liters per minute (L/min) of air is inhaled by the average person at rest, while 19.5 L/min is inhaled under conditions of light work [ICRP 1975]. Assuming that all of the air an employee inhales contains the concentration of diacetyl measured in the workplace, it is critical to recognize that the mainstream smoke of a smoker is not the only source of air; they are also breathing air with much lower diacetyl concentrations. Assuming that the other air breathed by smokers does not contain diacetyl, the workplace equivalent exposure concentration for a smoker during the smoking time period is roughly 190- to 560-fold lower than the concentration measured in the mainstream cigarette smoke using the ISO parameters of one 0.035 L puff/min (6.75/0.035 is 193 and 19.5/.035 is 557). Using the ISO parameters, this would be an average equivalent of a workplace concentration of 0.45 to 1.3 ppm for diacetyl and 58 to 170 ppb for 2,3-pentanedione during the smoking process. If the smoking machine parameters of 9.3 minutes/cigarette are used to calculate the duration of exposure, a smoker who smokes one pack of 20 cigarettes/day spends 186 minutes a day smoking, but the employee is working for 8 hours. Therefore,

the 8-hour time-weighted average equivalent concentration for the smoker is 2.6-fold lower to reflect the time period they are not exposed, which would be 170 to 500 ppb for diacetyl and 22 to 65 ppb for 2,3-pentanedione, depending upon exercise level.

Calculations of workplace equivalent exposures are similar using total mass of diacetyl and 2,3-pentanedione reported by Pierce et al. [Pierce et al. 2014]. The total mass of diacetyl and 2,3-pentanedione in an average cigarette measured with ISO parameters was 285 and 42.8 µg, respectively [Pierce et al. 2014]. For a one pack a day smoker that results in 5,700 µg of diacetyl and 856 µg of 2,3-pentanedione inhaled each day. If that mass was contained in the amount of air inhaled in a work day under light exercise conditions, at 25°C and 760 torr, the one pack/day smoker inhales the approximate equivalent of an occupational exposure to 169 ppb diacetyl and 22 ppb 2,3-pentanedione during an 8-hour work day.

Although it is important to recognize that breathing patterns differ between cigarette smokers or electronic cigarette users ("vapers") and employees, which may affect the pharmacokinetics of inhaled diacetyl and 2,3-pentanedione [Hubbs et al. 2015], these concentrations of diacetyl would be predicted to decrease FEV_1 in some individuals if inhaled for a working lifetime (see Chapter 5). Indeed, many smokers do demonstrate significant decreases in FEV_1 [Barnes 2004; Fletcher and Peto 1977; Wright et al. 1987; Xu et al. 1994]. Additionally, cigarette smoking is a major risk factor for chronic obstructive pulmonary disease, and a decrease in FEV_1 is a characteristic feature of this disease. In addition, bronchiolar fibrosis is part of the airway remodeling response that characterizes chronic obstructive pulmonary disease [Kim et al. 2008; Sohal et al. 2013]. Decreases in FEV_1 and fibrosis of bronchioles are features that also characterize obliterative bronchiolitis [Schlesinger et al. 1998]. However, smokers with chronic obstructive pulmonary disease have additional morphologic changes in their lungs, including emphysema, that are not seen in obliterative bronchiolitis [Snider 2003]. While it has been hypothesized that the failure to diagnose diacetyl-induced obliterative bronchiolitis as a cigarette smoker-associated disease suggests that diacetyl does not cause obliterative bronchiolitis in exposed employees [Pierce et al. 2014], the data when corrected for the actual corresponding occupational exposure concentrations may instead suggest the hypothesis that diacetyl and related reactive carbonyl compounds in cigarettes could potentially contribute to chronic obstructive pulmonary disease. Because chronic obstructive pulmonary disease is a leading cause of morbidity and mortality [Halbert et al. 2006; Lopez et al. 2006], the role of diacetyl and other reactive carbonyl compounds in cigarette smoke in contributing to chronic obstructive pulmonary disease is a worthy topic for future studies. As extensively discussed in Chapter 3, airway obstruction and decreased FEV_1 can, nevertheless, be identified in smokers who are exposed to diacetyl. Most importantly, because diacetyl causes obstructive lung disease and because smoking causes obstructive lung disease, the presence of diacetyl in cigarette smoke does not diminish the need to control diacetyl exposures in employees. In fact, it highlights the greater risks occurring in employees who are exposed to diacetyl in the workplace and who also smoke.

4.2.7 Relevance of Diacetyl Animal Studies to Humans

Four converging lines of evidence support the relevance of diacetyl inhalation studies in rats and mice to humans. First, diacetyl inhalation causes damage to respiratory epithelium in rats and mice. This finding is important because injury to the respiratory epithelium of the deep lung is the accepted cause for obliterative bronchiolitis. Second, dosimetry calculations indicate that diacetyl

concentration in respiratory epithelium of the human deep lung under working conditions may be much higher than diacetyl concentrations in laboratory animals. Third, another organic compound, sulfur mustard, implicated in causing obliterative bronchiolitis in humans, produces a similar pattern of predominantly nasal injury in rats exposed by nose-only inhalation [Weber et al. 2010]. Fourth, repeated inhalation exposure to 2,3-pentanedione causes fibrosis of intrapulmonary airways [Morgan et al. 2012; Morgan et al. 2016]. Each of these findings supports the conclusion that with appropriate dosimetry studies, damage to the respiratory epithelium of the upper airways of rodents should be considered when evaluating risk for 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. Importantly, diacetyl exposures in rodents caused extensive damage to the respiratory epithelium lining the nose and the trachea [Hubbs et al. 2008; Morgan et al. 2008]. The cell types that are injured in the nose and trachea in rodents are very similar to the respiratory epithelium lining the airways of the deep lung of humans that are involved pathophysiologically in the development of obliterative bronchiolitis [Borthwick et al. 2009; King 1989]. In addition, the bronchi were damaged at high concentrations in acute exposures and at lower concentrations in subchronic exposures in mice. Thus, inhalation toxicology studies showed that diacetyl could damage respiratory epithelium, providing biological plausibility for its etiologic role in obliterative bronchiolitis. Indeed, at the time of the first inhalation toxicology studies of diacetyl, no accepted cause of obliterative bronchiolitis in humans had been demonstrated to cause obliterative bronchiolitis in rodents. Recently, repeated inhalation exposures to 2,3-pentanedione have been shown to cause fibrosis of intrapulmonary airways in rats, demonstrating a pathologic change in the rodent model that is very similar to obliterative bronchiolitis in humans [Morgan et al. 2012]. Interpretation of the species difference in the anatomic location of diacetyl-induced damage to respiratory epithelium may be explained by species differences in respiratory tract anatomy, breathing patterns, and diacetyl dosimetry.

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 that 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 obliterative bronchiolitis [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 humans, 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 employees. 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 CFD-PBK model indicates that with low levels of exercise that could occur in the workplace, diacetyl dose to the bronchiolar epithelium of humans may be more than 40-fold greater than the dose received by the bronchiolar epithelium of experimentally exposed rodents [Gloede et al. 2011].

Damage to the nose of rodents has recently been described for another agent implicated in causing obliterative bronchiolitis in humans, sulfur mustard [bis(2-chloroethyl) sulfide], a chemical warfare agent. Obliterative bronchiolitis is considered a major cause of progressive respiratory disease in survivors of sulfur mustard exposure [Ghanei 2004a,b,c; Ghanei et al. 2008; Rowell et al. 2009]. Nose-only inhalation exposures of F344 rats to sulfur mustard caused severe mucosal damage in the rat nose but the changes in the lung were absent or minimal [Weber et al. 2010]. When the nose was bypassed using intubation with tubing lined by Teflon®, sulfur mustard did indeed cause necrosis of the epithelium lining the proximal airways [Weber et al. 2010]. This suggests that sulfur mustard, an accepted cause of obliterative bronchiolitis in humans, causes a similar pattern of injury to the pattern observed with diacetyl at different levels of the respiratory tract of rodents. Thus, predominantly nasal injury has been seen in rodent inhalation studies with organic agents implicated in causing obliterative bronchiolitis.

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 α-diketone, 2,3-pentanedione, suggest that the airway toxicity of diacetyl may be shared with other structurally related, α-diketones, and that inhalation of either diacetyl and 2,3-pentanedione can cause airway fibrosis in rats.

This page intentionally left blank.

References

Aeschbacher HU, Wolleb U, Loliger J, Spadone JC, Liardon R [1989]. Contribution of coffee aroma constituents to the mutagenicity of coffee. Food Chem Toxicol *27*(4):227–232.

Allen JG, Flanigan SS, LeBlanc M, Vallarino J, MacNaughton P, Stewart JH, Christiani DC [2016]. Flavoring chemicals in e-cigarettes: diacetyl, 2,3-pentanedione, and acetoin in a sample of 51 products, including fruit-, candy-, and cocktail-flavored e-cigarettes. Environ Health Perspect *124*(6):733–739.

Anderson SE, Franko J, Wells JR, Lukomska E, Meade BJ [2013]. Evaluation of the hypersensitivity potential of alternative butter flavorings. Food Chem Toxicol *62C*:373–381.

Anderson SE, Wells JR, Fedorowicz A, Butterworth LF, Meade BJ, Munson AE [2007]. Evaluation of the contact and respiratory sensitization potential of volatile organic compounds generated by simulated indoor air chemistry. Toxicol Sci *97*(2):355–363.

Barnes PJ [2004]. Mediators of chronic obstructive pulmonary disease. Pharmacol Rev *56*(4):515–548.

Borthwick LA, Parker SM, Brougham KA, Johnson GE, Gorowiec MR, Ward C, Lordan JL, Corris PA, Kirby JA, Fisher AJ [2009]. Epithelial to mesenchymal transition (EMT) and airway remodelling after human lung transplantation. Thorax *64*(9):770–777.

Boylstein R, Piacitelli C, Grote A, Kanwal R, Kullman G, Kreiss K [2006]. Diacetyl emissions and airborne dust from butter flavorings used in microwave popcorn production. J Occup Environ Hyg *3*(10):530–535.

Carbone V, Ishikura S, Hara A, El-Kabbani O [2005]. Structure-based discovery of human L-xylulose reductase inhibitors from database screening and molecular docking. Bioorg Med Chem *13*(2):301–312.

CDC [2013]. Obliterative bronchiolitis in workers in a coffee-processing facility—Texas, 2008-2012. MMWR *62*(16):305–307.

Cho-Vega JH, Tsavachidis S, Do KA, Nakagawa J, Medeiros LJ, McDonnell TJ [2007a]. Dicarbonyl/L-xylulose reductase: a potential biomarker identified by laser-capture microdissection-micro serial analysis of gene expression of human prostate adenocarcinoma. Cancer Epidemiol Biomarkers Prev *16*(12):2615–2622.

Cho-Vega JH, Vega F, Schwartz MR, Prieto VG [2007b]. Expression of dicarbonyl/L-xylulose reductase (DCXR) in human skin and melanocytic lesions: morphological studies supporting cell adhesion function of DCXR. J Cutan Pathol *34*(7):535–542.

Chuang LF, Collins EB [1968]. Biosynthesis of diacetyl in bacteria and yeast. J Bacteriol *95*(6):2083–2089.

Colley J, Gaunt IF, Lansdown AB, Grasso P, Gangolli SD [1969]. Acute and short-term toxicity of diacetyl in rats. Food Cosmet Toxicol *7*(6):571–580.

Conolly RB, Kimbell JS, Janszen D, Schlosser PM, Kalisak D, Preston J, Miller FJ [2004]. Human respiratory tract cancer risks of inhaled formaldehyde: dose-response predictions derived from biologically-motivated computational modeling of a combined rodent and human dataset. Toxicol Sci *82*(1):279–296.

Daglia M, Papetti A, Aceti C, Sordelli B, Spini V, Gazzani G [2007a]. Isolation and determination of α-dicarbonyl compounds by RP-HPLC-DAD in green and roasted coffee. J Agric Food Chem *55*(22):8877–8882.

Daglia M, Papetti A, Grisoli P, Aceti C, Spini V, Dacarro C, Gazzani G [2007b]. Isolation, identification, and quantification of roasted coffee antibacterial compounds. J Agric Food Chem *55*(25):10208–10213.

El-Kabbani O, Carbone V, Darmanin C, Ishikura S, Hara A [2005]. Structure of the tetrameric form of human L-xylulose reductase: probing the inhibitor-binding site with molecular modeling and site-directed mutagenesis. Proteins *60*(3):424–432.

Endo S, Matsunaga T, Horie K, Tajima K, Bunai Y, Carbone V, El-Kabbani O, Hara A [2007]. Enzymatic characteristics of an aldo-keto reductase family protein (AKR1C15) and its localization in rat tissues. Arch Biochem Biophys *465*(1):136–147.

Epperly BR, Dekker EE [1989]. Inactivation of Escherichia coli L-threonine dehydrogenase by

2,3-butanedione. Evidence for a catalytically essential arginine residue. J Biol Chem *264*(31):18296–18301.

Eriksson O, Fontaine E, Bernardi P [1998]. Chemical modification of arginines by 2,3-butanedione and phenylglyoxal causes closure of the mitochondrial permeability transition pore. J Biol Chem *273*(20):12669–12674.

Farsalinos KE, Kistler KA, Gillman G, Voudris V [2015]. Evaluation of electronic cigarette liquids and aerosol for the presence of selected inhalation toxins. Nicotine Tob Res *17*(2):168–174.

FASEB, Life Science Resource Office (Federation of American Societies for Experimental Biology) [1980]. Evaluation of the health aspects of starter distillate and diacetyl and food ingredients. Food and Drug Administration.

FDA (Food and Drug Administration) [1983]. Code of Federal Regulations, Title 21, Volume 3, Part 184, Direct food substances affirmed as generally recognized as safe, 21CFR184.1848, starter distillate. U.S. Government Printing Office.

Fedan JS, Dowdy JA, Fedan KB, Hubbs AF [2006]. Popcorn worker's lung: in vitro exposure to diacetyl, an ingredient in microwave popcorn butter flavoring, increases reactivity to methacholine. Toxicol Appl Pharmacol *215*(1):17–22.

Fedan JS, Millecchia LL, Johnston RA, Rengasamy A, Hubbs A, Dey RD, Yuan LX, Watson D, Goldsmith WT, Reynolds JS, Orsini L, Dortch-Carnes J, Cutler D, Frazer DG (485) [2000]. Effect of ozone treatment on airway reactivity and epithelium-derived relaxing factor in guinea pigs. J Pharmacol Exp Ther *293*(3):724–734.

Fletcher C, Peto R [1977]. The natural history of chronic airflow obstruction. Br Med J *1*(6077):1645–1648.

Flynn FV [1955]. Essential pentosuria. Br Med J *1*(4910):391–395.

Foster WM, Walters DM, Longphre M, Macri K, Miller LM [2001]. Methodology for the measurement of mucociliary function in the mouse by scintigraphy. J Appl Physiol *90*(3):1111–1117.

Frederick CB, Bush ML, Lomax LG, Black KA, Finch L, Kimbell JS, Morgan KT, Subramaniam RP, Morris JB, Ultman JS [1998]. Application of a hybrid computational fluid dynamics and physiologically based inhalation model for interspecies dosimetry extrapolation of acidic vapors in the upper airways. Toxicol Appl Pharmacol *152*(1):211–231.

Fujioka K, Shibamoto T [2006]. Determination of toxic carbonyl compounds in cigarette smoke. Environ Toxicol *21*(1):47–54.

Garcia-Quintans N, Repizo G, Martin M, Magni C, Lopez P [2008]. Activation of the diacetyl/acetoin pathway in Lactococcus lactis subsp. lactis bv. diacetylactis CRL264 by acidic growth. Appl Environ Microbiol *74*(7):1988–1996.

Ghanei M, Fathi H, Mohammad MM, Aslani J, Nematizadeh F [2004a]. Long-term respiratory disorders of claimers with subclinical exposure to chemical warfare agents. Inhal Toxicol *16*(8):491–495.

Ghanei M, Mokhtari M, Mohammad MM, Aslani J [2004b]. Bronchiolitis obliterans following exposure to sulfur mustard: chest high resolution computed tomography. Eur J Radiol *52*(2):164–169.

Ghanei M, Tazelaar HD, Chilosi M, Harandi AA, Peyman M, Akbari HM, Shamsaei H, Bahadori M, Aslani J, Mohammadi A [2008]. An international collaborative pathologic study of surgical lung biopsies from mustard gas-exposed patients. Respir Med *102*(6):825–830.

Gloede E, Cichocki JA, Baldino JB, Morris JB [2011]. A validated hybrid computational fluid dynamics-physiologically based pharmacokinetic model for respiratory tract vapor absorption in the human and rat and its application to inhalation dosimetry of diacetyl. Toxicol Sci *123*(1):231–246.

Goravanahally MP, Hubbs AF, Fedan JS, Kashon ML, Battelli LA, Mercer RR, Goldsmith WT, Jackson MC, Cumpston A, Frazer DG, Dey RD [2014]. Diacetyl increases sensory innervation and substance P production in rat trachea. Toxicol Pathol *42*(3):582–590.

Halbert RJ, Natoli JL, Gano A, Badamgarav E, Buist AS, Mannino DM [2006]. Global burden of COPD: systematic review and meta-analysis. Eur Respir J *28*(3):523–532.

Hubbs AF, Battelli LA, Goldsmith WT, Porter DW, Frazer D, Friend S, Schwegler-Berry D, Mercer RR, Reynolds JS, Grote A, Castranova V, Kullman G, Fedan JS, Dowdy J, Jones WG [2002]. Necrosis of nasal and airway epithelium in rats inhaling vapors of artificial butter flavoring. Toxicol Appl Pharmacol *185*(2):128–135.

Hubbs AF, Cummings KJ, McKernan LT, Dankovic DA, Park RM, Kreiss K [2015]. Comment on Farsalinos et al., "Evaluation of electronic cigarette liquids and

vapour for the presence of selected inhalation toxins." Nicotine Tob Res *17*(10):1288–1289.

Hubbs AF, Cumpston AM, Goldsmith WT, Battelli LA, Kashon ML, Jackson MC, Frazer DG, Fedan JS, Goravanahally MP, Castranova V, Kreiss K, Willard PA, Friend S, Schwegler-Berry D, Fluharty KL, Sriram K [2012]. Respiratory and olfactory cytotoxicity of inhaled 2,3-pentanedione in Sprague-Dawley rats. Am J Pathol *181*(3):829–844.

Hubbs AF, Fluharty KL, Edwards RJ, Barnabei JL, Grantham JT, Palmer SM, Kelly F, Sargent LM, Reynolds SH, Mercer RR, Goravanahally MP, Kashon ML, Honaker JC, Jackson MC, Cumpston AM, Goldsmith WT, McKinney W, Fedan JS, Battelli LA, Munro T, Bucklew-Moyers W, McKinstry K, Schwegler-Berry D, Friend S, Knepp AK, Smith SL, Sriram K [2016]. Accumulation of ubiquitin and sequestosome-1 implicate protein damage in diacetyl-induced cytotoxicity. Am J Pathol *186*(11):2887–2908.

Hubbs AF, Goldsmith WT, Kashon ML, Frazer D, Mercer RR, Battelli LA, Kullman GJ, Schwegler-Berry D, Friend S, Castranova V [2008]. Respiratory toxicologic pathology of inhaled diacetyl in Sprague-Dawley rats. Toxicol Pathol *36*(2):330–344.

ICRP [1975]. Report of the task group on reference man. Oxford: Pergamon Press. International Commission on Radiological Protection, ICRP Publication No. 23.

Ishikura S, Isaji T, Usami N, Kitahara K, Nakagawa J, Hara A [2001]. Molecular cloning, expression and tissue distribution of hamster diacetyl reductase. Identity with L-xylulose reductase. Chem Biol Interact *130–132*(1–3):879–889.

Jang HW, Jiang Y, Hengel M, Shibamoto T [2013]. Formation of 4(5)-methylimidazole and its precursors, α-dicarbonyl compounds, in Maillard model systems. J Agric Food Chem *61*(28):6865–6872.

Jiang Y, Hengel M, Pan C, Seiber JN, Shibamoto T [2013]. Determination of toxic α-dicarbonyl compounds, glyoxal, methylglyoxal, and diacetyl, released to the headspace of lipid commodities upon heat treatment. J Agric Food Chem *61*(5):1067–1071.

Johnson BA, Arguello S, Leon M [2007]. Odorants with multiple oxygen-containing functional groups and other odorants with high water solubility preferentially activate posterior olfactory bulb glomeruli. J Comp Neurol *502*(3):468–482.

Kelly FL, Sun J, Fischer BM, Voynow JA, Kummarapurugu AB, Zhang HL, Nugent JL, Beasley RF, Martinu T, Gwinn WM, Morgan DL, Palmer SM [2014]. Diacetyl induces amphiregulin shedding in pulmonary epithelial cells and in experimental bronchiolitis obliterans. Am J Respir Cell Mol Biol *51*(4):568–574.

Kim SB, Hayase F, Kato H [1987]. Desmutagenic effect of α-dicarbonyl and α-hydroxycarbonyl compounds against mutagenic heterocyclic amines. Mutat Res *177*(1):9–15.

Kim V, Rogers TJ, Criner GJ [2008]. New concepts in the pathobiology of chronic obstructive pulmonary disease. Proc Am Thorac Soc *5*(4):478–485.

King TE, Jr. [1989]. Bronchiolitis obliterans. Lung *167*(2):69–93.

Kullman G, Boylstein R, Jones W, Piacitelli C, Pendergrass S, Kreiss K [2005]. Characterization of respiratory exposures at a microwave popcorn plant with cases of bronchiolitis obliterans. J Occup Environ Hyg *2*(3):169–178.

Lane AB, Jenkins T [1985]. Human L-xylulose reductase variation: family and population studies. Ann Hum Genet *49*(Pt 3):227–235.

Larsen ST, Alarie Y, Hammer M, Nielsen GD [2009]. Acute airway effects of diacetyl in mice. Inhal Toxicol *21*(13):1123–1128.

Liggett RE, Drake MA, Delwiche JF [2008]. Impact of flavor attributes on consumer liking of Swiss cheese. J Dairy Sci *91*(2):466–476.

Lizarraga I, Alfaro MJ, Goicoechea C, Lopez F, Martin MI (1275) [1998]. Effect of butanedione monoxime on the contractility of guinea pig ileum and on the electrophysiological activity of myenteric S-type neurones. Neurosci Lett *246*(2):105–108.

Lopez AD, Shibuya K, Rao C, Mathers CD, Hansell AL, Held LS, Schmid V, Buist S [2006]. Chronic obstructive pulmonary disease: current burden and future projections. Eur Respir J *27*(2):397–412.

Majcher MA, Jelen HH [2005]. Identification of potent odorants formed during the preparation of extruded potato snacks. J Agric Food Chem *53*(16):6432–6437.

Majcher MA, Klensporf-Pawlik D, Dziadas M, Jelen HH [2013]. Identification of aroma active compounds of cereal coffee brew and its roasted ingredients. J Agric Food Chem *61*(11):2648–2654.

Marnett LJ, Hurd HK, Hollstein MC, Levin DE, Esterbauer H, Ames BN [1985]. Naturally occurring carbonyl compounds are mutagens in Salmonella tester strain TA104. Mutat Res *148*(1–2):25–34.

Mathews JM, Watson SL, Snyder RW, Burgess JP, Morgan DL [2010]. Reaction of the butter flavorant diacetyl (2,3-butanedione) with N-α-acetylarginine: a model for epitope formation with pulmonary proteins in the etiology of obliterative bronchiolitis. J Agric Food Chem *58*(24):12761–12768.

Mercer RR, Russell ML, Roggli VL, Crapo JD [1994]. Cell number and distribution in human and rat airways. Am J Respir Cell Mol Biol *10*(6):613–624.

Miller AG, Gerrard JA [2005]. Assessment of protein function following cross-linking by alpha-dicarbonyls. Ann N Y Acad Sci Jun; *1043*:195–200.

More SS, Raza A, Vince R [2012a]. The butter flavorant, diacetyl, forms a covalent adduct with 2-deoxyguanosine, uncoils DNA, and leads to cell death. J Agric Food Chem *60*(12):3311–3317.

More SS, Vartak AP, Vince R [2012b]. The butter flavorant, diacetyl, exacerbates β-amyloid cytotoxicity. Chem Res Toxicol *25*(10):2083–2091.

Morgan DL, Flake GP, Kirby PJ, Palmer SM [2008]. Respiratory toxicity of diacetyl in C57BL/6 mice. Toxicol Sci *103*(1):169–180.

Morgan DL, Jokinen MP, Johnson CL, Price HC, Gwinn WM, Bousquet RW, Flake GP [2016]. Chemical reactivity and respiratory toxicity of the alpha-diketone flavoring agents: 2,3-butanedione, 2,3-pentanedione, and 2,3-hexanedione. Toxicol Pathol *44*(5):763–783.

Morgan DL, Jokinen MP, Price HC, Gwinn WM, Palmer SM, Flake GP [2012]. Bronchial and bronchiolar fibrosis in rats exposed to 2,3-pentanedione vapors: implications for bronchiolitis obliterans in humans. Toxicol Pathol *40*(3):448–465.

Morgan DL, Merrick BA, Gerrish KE, Stockton PS, Wang Y, Foley JF, Gwinn WM, Kelly FL, Palmer SM, Ton TV, Flake GP [2015]. Gene expression in obliterative bronchiolitis-like lesions in 2,3-pentanedione-exposed rats. PLoS One *10*(2):e0118459.

Morris JB [1997]. Uptake of acetaldehyde vapor and aldehyde dehydrogenase levels in the upper respiratory tracts of the mouse, rat, hamster, and guinea pig. Fundam Appl Toxicol *35*(1):91–100.

Morris JB, Hubbs AF [2009]. Inhalation dosimetry of diacetyl and butyric acid, two components of butter flavoring vapors. Toxicol Sci *108*(1):173–183.

Nakagawa J, Ishikura S, Asami J, Isaji T, Usami N, Hara A, Sakurai T, Tsuritani K, Oda K, Takahashi M, Yoshimoto M, Otsuka N, Kitamura K [2002a]. Molecular characterization of mammalian dicarbonyl/L-xylulose reductase and its localization in kidney. J Biol Chem *277*(20):17883–17891.

Nakagawa J, Oide K, Uematsu Y, Sudo T, Sakurai T, Asami J, Tada N, Ishikura S, Hara A, Odani H, Maeda K [2002b]. Characterization of a dicarbonyl/L-xylulose reductase (DCXR) in kidney with trangenic and knockout mice. Nephrol Dial Transplant *17*(S 12):52.

National Toxicology Program [2007]. Chemical information review document for artificial butter flavoring and constituents: diacetyl [CAS No. 431-03-8] and acetoin [CAS No. 513-86-0]. http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/Artificial_butter_flavoring.pdf. Date accessed: March 2011.

Otsuka M, Mine T, Ohuchi K, Ohmori S [1996]. A detoxication route for acetaldehyde: metabolism of diacetyl, acetoin, and 2,3-butanediol in liver homogenate and perfused liver of rats. J Biochem (Tokyo) *119*(2):246–251.

Palmer SM, Flake GP, Kelly FL, Zhang HL, Nugent JL, Kirby PJ, Foley JF, Gwinn WM, Morgan DL [2011]. Severe airway epithelial injury, aberrant repair and bronchiolitis obliterans develops after diacetyl instillation in rats. PLoS One *6*(3):e17644.

Pierce JS, Abelmann A, Spicer LJ, Adams RE, Finley BL [2014]. Diacetyl and 2,3-pentanedione exposures associated with cigarette smoking: implications for risk assessment of food and flavoring workers. Crit Rev Toxicol *44*(5):420–435.

Polzin GM, Kosa-Maines RE, Ashley DL, Watson CH [2007]. Analysis of volatile organic compounds in mainstream cigarette smoke. Environ Sci Technol *41*(4):1297–1302.

Rao GV, Tinkle S, Weissman DN, Antonini JM, Kashon ML, Salmen R, Battelli LA, Willard PA, Hoover MD, Hubbs AF [2003]. Efficacy of a technique for exposing the mouse lung to particles aspirated from the pharynx. J Toxicol Environ Health A *66*(15):1441–1452.

Rigler MW, Longo WE [2010]. Emission of diacetyl (2,3 butanedione) from natural butter, microwave popcorn

butter flavor powder, paste, and liquid products. Int J Occup Environ Health *16*(3):291–302.

Rincon-Delgadillo MI, Lopez-Hernandez A, Wijaya I, Rankin SA [2012]. Diacetyl levels and volatile profiles of commercial starter distillates and selected dairy foods. J Dairy Sci *95*(3):1128–1139.

Roberts DW, York M, Basketter DA [1999]. Structure-activity relationships in the murine local lymph node assay for skin sensitization: α,β-diketones. Contact Dermatitis *41*(1):14–17.

Rowell M, Kehe K, Balszuweit F, Thiermann H [2009]. The chronic effects of sulfur mustard exposure. Toxicology *263*(1):9–11.

Santos CM, Valente IM, Goncalves LM, Rodrigues JA [2013]. Chromatographic analysis of methylglyoxal and other α-dicarbonyls using gas-diffusion microextraction. Analyst *138*(23):7233–7237.

Saraiva MA, Borges CM, Florencio MH [2006]. Non-enzymatic model glycation reactions: a comprehensive study of the reactivity of a modified arginine with aldehydic and diketonic dicarbonyl compounds by electrospray mass spectrometry. J Mass Spectrom *41*(6):755–770.

Sawada H, Hara A, Nakayama T, Seiriki K [1985]. Kinetic and structural properties of diacetyl reductase from hamster liver. J Biochem *98*(5):1349–1357.

Schlesinger C, Meyer CA, Veeraraghavan S, Koss MN [1998]. Constrictive (obliterative) bronchiolitis: diagnosis, etiology, and a critical review of the literature. Ann Diagn Pathol *2*(5):321–334.

Siegman MJ, Mooers SU, Warren TB, Warshaw DM, Ikebe M, Butler TM (1277) [1994]. Comparison of the effects of 2,3-butanedione monoxime on force production, myosin light chain phosphorylation and chemical energy usage in intact and permeabilized smooth and skeletal muscles. J Muscle Res Cell Motil *15*(4):457–472.

Snider GL [2003]. Nosology for our day: its application to chronic obstructive pulmonary disease. Am J Respir Crit Care Med *167*(5):678–683.

Sohal SS, Ward C, Danial W, Wood-Baker R, Walters EH [2013]. Recent advances in understanding inflammation and remodeling in the airways in chronic obstructive pulmonary disease. Expert Rev Respir Med *7*(3):275–288.

Speckman RA, Collins EB [1968]. Diacetyl biosynthesis in Streptococcus diacetilactis and Leuconostoc citrovorum. J Bacteriol *95*:174–180.

St-Gelais D, Lessard J, Champagne CP, Vuillemard JC [2009]. Production of fresh cheddar cheese curds with controlled postacidification and enhanced flavor. J Dairy Sci *92*(5):1856–1863.

Stowe DF, O'Brien WC, Chang D, Knop CS, Kampine JP (1276) [1997]. Reversal of endothelin-induced vasoconstriction by endothelium-dependent and -independent vasodilators in isolated hearts and vascular rings. J Cardiovasc Pharmacol *29*(6):747–754.

Sugai S, Murata K, Kitagaki T, Tomita I [1990]. Studies on eye irritation caused by chemicals in rabbits–1. A quantitative structure-activity relationships approach to primary eye irritation of chemicals in rabbits. J Toxicol Sci *15*(4):245–262.

Sumegi B, Gyocsi L, Alkonyi [1982]. Diacetyl: a new substrate in the overall reaction of the pyruvate dehydrogenase complex. Biochim Biophys Acta *705*(1):70–75.

van Rooy FG, Rooyackers JM, Prokop M, Houba R, Smit LA, Heederik DJ [2007]. Bronchiolitis obliterans syndrome in chemical workers producing diacetyl for food flavorings. Am J Respir Crit Care Med *176*(5):498–504.

Waurick R, Knapp J, Van Aken H, Boknik P, Neumann J, Schmitz W (1277) [1999]. Effect of 2,3-butanedione monoxime on force of contraction and protein phosphorylation in bovine smooth muscle. Naunyn Schmiedebergs Arch Pharmacol *359*(6):484–492.

Weber WM, Kracko DA, Lehman MR, Irvin CM, Blair LF, White RK, Benson JM, Grotendorst GR, Cheng YS, McDonald JD [2010]. Inhalation exposure systems for the development of rodent models of sulfur mustard-induced pulmonary injury. Toxicol Mech Methods *20*(1):14–24.

Whittaker P, Clarke JJ, San RH, Begley TH, Dunkel VC [2008]. Evaluation of the butter flavoring chemical diacetyl and a fluorochemical paper additive for mutagenicity and toxicity using the mammalian cell gene mutation assay in L5178Y mouse lymphoma cells. Food Chem Toxicol *46*(8):2928–2933.

Wondrak GT, Cervantes-Laurean D, Roberts MJ, Qasem JG, Kim M, Jacobson EL, Jacobson MK [2002]. Identification of α-dicarbonyl scavengers for cellular protection against carbonyl stress. Biochem Pharmacol *63*(3):361–373.

Wright JL, Hobson J, Wiggs BR, Pare PD, Hogg JC [1987]. Effect of cigarette smoking on structure of the small airways. Lung *165*(2):91–100.

Xu X, Weiss ST, Rijcken B, Schouten JP [1994]. Smoking, changes in smoking habits, and rate of decline in FEV1: new insight into gender differences. Eur Respir J *7*(6):1056–1061.

Zaccone EJ, Thompson JA, Ponnoth DS, Cumpston AM, Goldsmith WT, Jackson MC, Kashon ML, Frazer DG, Hubbs AF, Shimko MJ, Fedan JS [2013]. Popcorn flavoring effects on reactivity of rat airways in vivo and in vitro. J Toxicol Environ Health A, Part A *76*:669–689.

Zhou Y, Lee J-Y, Lee C-M, Cho W-K, Kang M-J, Koff JL, Yoon P-O, Chae J, Park H-O, Elias JA [2012]. Amphiregulin, an epidermal growth factor receptor ligand, plays an essential role in the pathogenesis of transforming growth factor-β-induced pulmonary fibrosis. J Biol Chem *287*(50):41991–42000.

5 Quantitative Risk Assessment Based on Employee Data

Taken together, the human and animal studies provide a compelling case for the respiratory toxicity of diacetyl and 2,3-pentanedione, and potentially other alpha diketones used in butter flavorings, of which diacetyl is the most thoroughly studied. The clinical experience and employee population studies have revealed a clear association between diacetyl exposure and diminishing respiratory capacity that has been shown in some cases to become manifest as obliterative bronchiolitis. The expanding animal research on diacetyl clearly describes pathological changes specific to this compound that provide an ample mechanistic basis for anticipating respiratory disease in humans. In this chapter, a risk assessment is presented that begins with the established premise that diacetyl causes irreversible respiratory damage. The analyses presented are designed specifically to describe that causal relationship for the purpose of predicting risk in working populations, not to prove that a causal relationship exists. Thus statistical significance is less important than insights provided into the nature of the relationship between diacetyl and diminishing respiratory capacity.

Other potentially reactive or toxic compounds can be present in association with diacetyl in flavoring applications, such as acetoin or acetaldehyde. An NTP 90-day study on acetoin is in progress [National Toxicology Program 2013b] but the chosen maximum exposure level (generally representing the maximum tolerated dose) is 800 ppm whereas in the NTP 90-day diacetyl study the maximum exposure level is 100 ppm [National Toxicology Program 2013a]. This implies a considerably lower level of potency for acetoin toxicity. Furthermore, in the population on which the risk assessment was based, acetoin concentrations were an order of magnitude lower than diacetyl levels. Acetaldehyde is less consistently associated with diacetyl and is often below the limit of detection.

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 statistical models that describe the relationship between exposure to diacetyl and the development of impaired lung function in a known population of exposed employees. Exposure-response modeling requires making assumptions about the exposures of the persons studied over the course of their working lifetime, and about the mathematical form of the exposure-response relationship. Using these models, a further goal was to estimate an exposure level below which there would be a relatively low risk. One approach that is used (benchmark dose) is to estimate what additional proportion of a known population would have abnormal lung function if their past exposure corresponded to a lifetime of working at some specific exposure level. Another approach estimates how many new cases of abnormal lung function would develop over a lifetime (excess lifetime risk) as a result of working at various exposure levels. Finally, the various methods are used to develop a range of plausible risk estimates for occupational exposure to diacetyl.

Although diacetyl causes obliterative bronchiolitis, 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. 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 of impairment (obstructive or restrictive or combined). American Thoracic Society/European Respiratory Society (ATS/ERS) recommendations are to use FEV_1 to assess the severity of any type of spirometric abnormality [Pellegrino et al. 2005]. The health effects outcomes of diacetyl exposure that NIOSH used in this 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 employees whose FEV_1 and/or FEV_1/FVC are below their *lower limits of normal*, conditions that plausibly would include cases of developing obliterative bronchiolitis.

5.1 Methods: Study Population, Exposure Assessment, and Outcomes

5.1.1 Study Population

Six NIOSH HHEs conducted at workplaces producing microwave popcorn with diacetyl exposures were reviewed for possible use in risk assessment [NIOSH 2003a, b, c, 2004a, b, 2006]. Two of the HHEs had very small workforces involved in popcorn production (< 10 per shift) [NIOSH 2003b, c]. 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

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

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

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 and area diacetyl air samples using NIOSH Method 2557. This sampling identified a number of air contaminants in addition to diacetyl and acetoin (Table 5-2), including acetaldehyde. 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. NIOSH researchers worked extensively to understand this problem and derive an appropriate correction for estimating diacetyl levels [Cox-Ganser et al. 2011]. This correction, based on absolute humidity and time to extraction, was applied to the diacetyl exposure levels above the limit of detection (LOD) as measured in the selected HHEs. For other chemical exposures in microwave popcorn production determined using NIOSH Method 2557, such as acetoin, no humidity/extraction correction was needed.

For laboratory diacetyl determinations below the 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 (corrected) was used. 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 had not 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 for diacetyl mixers, soon after the introduction of microwave production

Table 5-2. Numbers of air samples for diacetyl and acetoin 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	262	346	270	314

following an outbreak of eye irritation. NIOSH-measured diacetyl exposure levels for key process locations showed considerable variation across the four selected 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 situations at Companies G and N.

Mean diacetyl exposures for the Company K, L, and N populations were calculated classifying by department and job (Appendix G, Tables G.1–G.3) based on the corrected 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 based on their inquiries the HHE investigators determined that no significant control changes had been implemented prior to the first survey. For Company G, with repeated environmental assessments between November 2000 and July 2003 (2.7 years), to estimate employees' diacetyl exposures over time within department/job combinations, a job exposure matrix (JEM) was constructed through collaboration between NIOSH and OSHA (Appendix H). 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-corrected personal breathing zone sample concentrations (in parts per million), means were calculated for the cells in the JEM (Appendix G, Table G.4). Arithmetic means of personal samples are the preferred measure of central tendency for estimating cumulative exposure in

Table 5-3. Arithmetic mean air concentrations (ppm) of diacetyl in major processes at four sites

	Mixing		Production		Quality control		Maintenance	
Company	n, n_{ND}	Mean (SD)	n, n_{ND}	Mean (SD)	n, n_{ND}	Mean (SD)	n, n_{ND}	Mean (SD)
				Personal samples				
N	1,0	0.79 (0.00)	7,0	0.740 (0.640)	2,0	0.250 (0.014)	2,0	0.160 (0.066)
K	5,1	0.31 (0.41)	7,5	0.040 (0.079)	3,3	0.003 (0.001)	3,2	0.020 (0.030)
L	10,0	1.15 (0.74)	36,0	0.028 (0.016)	5,0	0.034 (0.019)	6,1	0.014 (0.008)
G	25,1	2.36 (3.92)	112,34	0.490 (0.900)	20,4	0.366 (0.390)	17,9	0.080 (0.126)
				Area samples				
N	2,0	1.03 (0.45)	2,0	0.620 (0.140)	2,0	0.320 (0.140)	0,0	—
K	2,1	2.42 (3.42)	7,5	0.032 (0.052)	3,3	0.002 (0.000)	2,1	0.037 (0.048)
L	6,0	1.54 (0.91)	23,0	0.028 (0.015)	6,0	0.018 (0.011)	3,0	0.019 (0.014)
G	50,1	16.8 (31.6)	123,29	1.050 (1.870)	24,10	0.253 (0.370)	17,11	0.122 (0.327)

Abbreviations: n = total number of samples, n_{ND} = number of non-detect samples, SD = standard deviation,—indicates lack of data
Note: Means are for corrected concentrations and given in parts per million (ppm); not all jobs fall within the four process categories displayed.

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

chronic disease investigations [Smith 1992]. However, for the first industrial hygiene survey (November 2000), only area samples were collected. For this survey, personal-sample equivalents to the area samples were estimated using area and personal sampling data from surveys 2 and 3 for the higher-exposed jobs, and using other procedures for samples with the lower values (Appendix H, Table H.3). 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. Exposure estimates in the JEM were assigned to employees based on their history of jobs performed, job 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 employees such as those in the mixers exposure category, the measured personal diacetyl exposure was adjusted for the use of respirators in selected exposure periods (Appendix H).

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. mist, 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 estimated to have first been 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 employees 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 employees except at Company G where, due to repeated pulmonary testing over months or years, initially current employees could become former employees 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 employee interview and consisted of

successive periods 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 PFT results defined the HHE outcomes. A medical questionnaire was administered that included standard ATS items on respiratory health [Ferris 1978] 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 1978]. The predicted and lower limit of normal (LLofN) values for FEV_1, FVC and FEV_1/FVC were calculated using prediction equations produced from NHANES III [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 nonsmoking general population classified by age, sex, race, and height.

For risk assessment purposes employees' percent of predicted values for FEV_1 (pp FEV_1) and actual FEV_1/FVC ratios were the outcomes modeled as continuous variables. In identifying possible developing obliterative bronchiolitis cases, a classification of pulmonary impairment was defined based on FEV_1 and/or FEV_1/FVC being less than their respective LLofN. This discrete outcome, onset of impairment, was analyzed by modeling incidence rates. Obliterative bronchiolitis 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, 57% of the cases defined using FEV_1 at Company G 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%). Thus 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 obliterative bronchiolitis cases is not routinely performed in NIOSH HHEs, but full disclosure of individual test results and recommendations for referral are provided to participating employees.

5.2 Methods: Analysis of Exposure Response

5.2.1 Exposure Metrics

The most appropriate measure of past diacetyl exposure for predicting health consequences is not known and hence was determined by assessment of the statistical fit of models using different exposure terms. Cumulative exposure (time summation of concentration, 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:

$$\text{cum}(DA) = \Sigma_i\,(DA),\ \text{cum}(DA^{0.5}) = \Sigma_i\,(DA^{0.5}),\ \text{and}\ \text{cum}(DA^{2.0}) = \Sigma_i\,(DA^{2.0})$$

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 available from full-shift (8-hour) TWA concentrations although selected jobs had been

analyzed using a real-time method (FTIR) to assess time-variability.

5.2.2 Models of Percent Predicted FEV_1 and FEV_1/FVC

The spirometry determinations, (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. Models of FEV_1/FVC included age (centered at 40). Known potential confounders were retained in models regardless of statistical significance according to good epidemiologic practice. Models were assessed using overall 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 or employment selection effects would be 100 (in nonsmokers). 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 employees with two or more spirometry results. All employees 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 employee's intercepts and exposure responses. A second set of metrics was calculated with exposure cumulation starting at the time of an employee's first survey and used in a subsidiary longitudinal analysis along with the full cumulative exposure metric. 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. A plant effect was introduced to allow for systematic differences between the two sites, and there was a test of heterogeneity in the exposure effects.

5.2.3 Models of the Incidence of Pulmonary Obstruction

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

(1) FEV_1 < LLofN; n=36

(2) FEV_1/FVC < LLofN; n=27

(3) FEV_1 < LLofN and FEV_1/FVC < LLofN; n=19

Definitions 2 and 3 represent definitions more specific to obstruction. For the combined Company K and L populations, the case definition used for determining onset of pulmonary impairment was: FEV_1 < LLofN (n=25). The definitions more specific to obstruction produced too few cases for meaningful analysis with the combined Company K and L populations.

In identifying cases, a date of onset for a condition resulting in impairment and possibly representing early obliterative bronchiolitis was estimated as the average of the dates on which the employee 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 their date of first exposure to diacetyl. The average date was chosen over the first date to be more robust for recalled dates. If no symptom date existed, then date of onset was set to the date of the first case-qualifying spirometry result (n=12, case definition 1; n=4, case definition 3 for Company G) unless this was the employee's first survey in which case the employee was excluded from analysis (n=42, case definition 1; n=21, case definition 3 for Company G). These excluded employees may have had onset of impairment prior to exposure but, according to the participating HHE clinicians, may also have included asymptomatic cases caused by diacetyl exposure with unknown onset date.

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 employees more often than others with similar exposure from employment. Incident cases 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 $\sqrt{Cum(DA)}$ ($p=4\times10^{-7}$) performing considerably better than exposure duration alone, and with average exposure to diacetyl [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 0.50 reduction in $ppFEV_1$ for each ppm-year of cumulative exposure (Tables 5-5, 5-6). (After 1 year at 1 ppm an employee's $ppFEV_1$, starting at 100, would be predicted to be 99.5.) For FEV_1/FVC the percent reduction with 1 ppm-yr DA was 0.16.

Seventy-nine percent of the cross-sectional study population (n=286) had duration of employment of < 4 yr and 49% had less than 6 months, reflecting a high workforce turnover rate. Models restricted to < 4 yr duration produced considerably larger effect estimates; for $ppFEV_1$: −1.07 (vs. −0.50) and for FEV_1/FVC: −0.87 (vs. −0.16) (Table 5-5). With < 4 yr, the $\sqrt{Cum(DA)}$ metric was a less strong predictor than Cum(DA).

In the models with the better predicting exposure metrics, gender and ethnicity (possible indicators of differential healthy employee 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

Table 5-5. Multiple regression models for percent predicted FEV_1 and FEV_1/FVC: various diacetyl exposure metrics for Company G

	Percent predicted FEV_1					FEV_1/FVC (expressed as percent)				
Exposure metric	**R^2**	**Int**	**est**	***t***	***P***	**R^2**	**Int**	**est**	***t***	***P***
					All (n=361)					
Avg(DA)	0.128	94.99	−1.77	2.41	0.0167	0.348	76.88	−1.26	−3.58	0.004
$Cum(DA^{2.0})$	0.142	94.62	−0.081	3.41	0.0007	0.338	76.19	−0.032	−2.77	0.0059
$(Cum(DA))^{2.0}$	0.148	94.76	−0.012	3.76	0.0002	0.334	76.08	−0.0036	−2.31	0.021
Duration	0.161	97.17	−0.964	4.43	9×10^{-6}	0.333	76.78	−0.256	−2.12	0.035
Cum(DA)	0.169	95.95	−0.500	4.83	10^{-6}	0.342	76.62	−0.164	−3.06	0.0024
$Cum(DA^{0.5})$	0.172	96.38	−0.843	4.95	7×10^{-7}	0.339	76.71	−0.258	−2.87	0.0044
$(Cum(DA))^{0.5}$	0.174	97.34	−2.77	5.04	4×10^{-7}	0.346	77.25	−0.981	−3.41	0.0007
$(Cum(DA^{0.5}))^{0.5}$	0.176	98.25	−3.70	5.16	2×10^{-7}	0.344	77.49	−1.24	−3.24	0.0013
					Less than 4 yr exposure duration (n=286)					
Cum(DA)	0.095	100.24	−1.069	−2.48	0.014	0.321	77.28	−0.872	−4.34	1.4×10^{-5}
$(Cum(DA))^{0.5}$	0.087	100.76	−2.57	−1.93	0.054	0.314	77.90	−2.47	−3.98	6×10^{-5}

Int = intercept; est = effect estimate for exposure metric; *t* = t-statistic for estimate; *P* = *P* value
Avg(DA) – time-weighted average exposure = cum(DA)/duration
Cum(DA) = cumulative exposure = Σ_i (DA)
$Cum(DA^{0.5}) = \Sigma_i (DA^{0.5})$
$Cum(DA^{2.0}) = \Sigma_i (DA^{2.0})$
Model = $ppFEV_1 = \alpha + \beta sex + \gamma Hispanic + \delta Black + \varepsilon smoker + \theta packyrs + \Phi packyrs^2 + \eta(exposure\ metric)$
Model = $FEV_1/FVC = \alpha + \beta sex + \beta Age + \gamma Hispanic + \delta Black + \varepsilon smoker + \theta packyrs + \Phi packyrs^2 + \eta(exposure\ metric)$

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	4×10^{-7}	−0.843	7×10^{-7}	−3.70	2×10^{-7}

probably negative—higher exposures have less than proportional association with decreases in spirometry. Results for Company N, based on a small number (n=35) of employees and only 20 breathing-zone air samples, 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 $\sqrt{Cum(DA)}$ were the better predictors of FEV_1/FVC; for Company K, Cum(DA) was best while for Company L, Avg(DA), $\sqrt{Cum(DA)}$ 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, Avg(DA) was the strongest predictor of $ppFEV_1$. In the pooled analysis of the Company K and Company L plants, the differences in exposure response (heterogeneity) between the plants for $ppFEV_1$ and FEV_1/FVC were highly significant for the better predicting metrics Cum(DA) and $(Cum(DA)^2$ (Tables 5-9, 5-10). The pooled regression estimate for the Cum(DA) metric corresponded to a decline 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 estimated decline in $ppFEV_1$ of 0.50 per ppm-yr of cumulative exposure (Table 5-6) but with very different estimates for the individual plants. For plant K, the estimated fall in $ppFEV_1$ per ppm-yr was 7.83 while for Plant L the decrease in $ppFEV_1$ was 2.70 (= −7.83+5.13) per ppm-yr. 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 had been given priority for control interventions, then the subsequently measured levels would underestimate most of the jobs previously 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 final basis for the NIOSH risk assessment for diacetyl which, instead, relied on the Company G findings.

By far the highest exposures at Company G were among mixers (Table 5-3) raising the possibility that the observed losses in pulmonary function could be limited to that group. To examine this question, the basic multiple regression models (Table 5-6) were applied to the population at Plant G from which all employees who were ever mixers had been excluded. The result was slightly stronger estimates of the DA effect both for (1) the linear cumulative exposure term (β=0.61 vs. 0.50 for full population; R^2=0.182 vs. 0.169, resp.) and (2) the square root of cumulative exposure (β=3.02 vs. 2.75 for full population; R^2=0.182 vs. 0.173, respectively) (results not shown).

Another concern was the possibility of a diacetyl-smoking interaction, with smoking possibly enhancing the harmful effect of diacetyl. Models for $ppFEV_1$ and FEV_1/FVC including interaction terms (products of the diacetyl exposure metrics with the ever-smoking and pack-yrs terms) yielded statistically significant *protective* effects of ever-smoking on the linear and square root cumulative diacetyl exposures and small, mostly insignificant, additive interactions for

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														
Company	**n**	***β***	***R²***	***P***	***β***	***R²***	***P***	***β***	***R²***	***P***	***β***	***R²***	***P***	***β***	***R²***	***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	0.00	.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 (expressed as percent)														
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

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 5-8. 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					
Company	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

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		Avg(DA)		$(Cum(DA))^{0.5}$		Cum(DA)		$(Cum(DA))^{2.0}$	
	$R^2 = 0.129$		$R^2 = 0.171$		$R^2 = 0.191$		$R^2 = 0.188$		$R^2 = 0.165$	
	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 (expressed as percent)

	Duration		Avg(DA)		$(Cum[DA])^{0.5}$		Cum(DA)		$(Cum[DA])^{2.0}$	
	$R^2 = 0.209$		$R^2 = 0.226$		$R^2 = 0.289$		$R^2 = 0.292$		$R^2 = 0.268$	
	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

the pack-years × diacetyl metric terms (*P* = 0.04 – 0.25 depending on exposure metric and outcome; data not shown). In the absence of the smoking interaction terms, the diacetyl effects in non-smokers are somewhat underestimated and overestimated in smokers.

Acetoin is another exposure in the popcorn flavoring environment (typically present with diacetyl in flavoring additive packages), and its presence was strongly associated with diacetyl (corr = 0.85) at Plant G, 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 inaccurate, NIOSH 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 employees' 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 0.1743 and 0.1737, respectively, for acetoin and diacetyl; the t-statistics for the exposure terms were 5.09 and 5.06 respectively. In microwave production jobs at Plant G, the mean DA concentration over all sampling surveys, combining both area and personal samples, was 3.4 ppm compared with 0.28 ppm for acetoin determinations from the same air samples. Because there is little support for acetoin itself playing a major role other than as surrogate for diacetyl in pulmonary toxicity, and because acetoin was present at much lower concentrations, this result supports the validity of the diacetyl exposure assessment and subsequent findings, but also implies that the diacetyl effects are being underestimated as a result of misclassification, otherwise the diacetyl effects would produce a stronger model fit than acetoin.

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 $\sqrt{\text{Cum(DA)}}$ exposure metrics (Table 5-11, models 1 and 2) compared to the analyses based on the FEV$_1$ at last survey (Tables 5-7, 5-8); the effects remain statistically significant. Differences in the effects of employees' exposures accruing from their initial evaluation (their first survey) until the current survey, compared to all exposures prior to the current survey, using either the metric Cum(DA) or $\sqrt{\text{Cum(DA)}}$, were small and not statistically significant ($P > 0.7$) (Table 5-11, models 3 and 4). This supports the conclusion that bias arising from cases preferentially leaving employment prior to the first survey is not different from that following the first survey when exposures were declining, suggesting that the bias in estimating the decline in ppFEV$_1$ is not large.

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). The original sentinel cases of obliterative bronchiolitis reported from this plant were not present in this study population. For the first definition of case (FEV$_1$< LLofN, n=36), excluding (a) candidate cases for which no qualifying date of onset was available and (b) subjects with missing smoking data, left 314 subjects for analysis. Increasing duration of exposure or diacetyl cumulative exposure (Cum(DA)) both predicted *diminishing* onset (Table 5-12, models 1 and 2). Model fit improved with both terms in the model but the duration effect remained negative. Other diacetyl metrics performed similarly (Table 5-12) with avg(DA) and cum(DA) providing the best fit (largest Δ-2lnL, smallest LRT *P* value). The negative duration term implies diminishing background rate with increasing duration.

Using case definition 2, (FEV$_1$/FVC< LLofN, n=27) the same pattern was observed, with the negative duration effect (P=0.0004) and positive cumulative exposure effect (P=.00003) now highly statistically significant despite a smaller number of cases (Table 5-13, model 3). With the most stringent case definition 3, (FEV$_1$< LLofN *and* FEV$_1$/FVC< LLofN), the negative duration effect (P=0.023) and the cumulative exposure effect (P=.016) remained statistically significant now with 19 cases (Table 5-14, model 3). In this model, smoking effects were not statistically significant, and age and sex were marginally significant (Table 5-15). Three other metrics yielded strong associations based on likelihood ratio test, particularly $\sqrt{\text{Cum(DA)}}$ and (Avg(DA)) (both P=.003) although with average exposure, duration was no longer significant (Table 5-14).

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 low-risk survivor population 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 years) 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 tenfold difference in cumulative exposure. Of the 36 cases, 22 occurred in the first 4 years of exposed employment, which encompassed about 80% of the study population. The rapid onset of this disease has been reported

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 an employee (two or more are in the analysis). Cum(DA) since first survey is calculated from an employee'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: exposure metrics (case definition 1: FEV_1 < LLofN; n=36)

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	0.92	0.96	0.0	0.14	—
2	Cum(DA)	−8.96	−0.0002	1.00	1.00	—	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

Baseline rate: as Log(rate); per day
LRT = likelihood ratio test
Model = rate = $\exp(\alpha + \beta smoker + \gamma sex + \delta(age - 40) + \varepsilon(age - 40)^2) + \theta packyrs + \Phi packyrs^2 + \eta cum(dur) + \mu cum(DA))$ (model 3)
RR = relative rate, at 1 or 2 ppm, or 1 year (duration)
Δ − 2lnL = change in − 2 × ln(likelihood), relative to model 1)

Table 5-13. Company G incidence rate models: exposure metrics (case definition 2: $FEV_1/FVC < LLofN$; n = 27)

Model no.	Metric	Intercept baseline rate	Effect estimate	RR 10yr @ 1 ppm	RR 5yr @ 2 ppm	Δ – 2lnL	Wald *P*	LRT *P*
1	Duration	−9.37	−0.081	—	—	—	0.20	—
2	Cum(DA)	−10.04	0.028	1.32	1.32	—	0.19	—
3	Duration	−9.54	−0.416				0.0004	
	Cum(DA)		0.140	4.06	4.06	14.55	0.00003	0.0001
4	Duration	−9.40	−0.972				0.0003	
	$Cum(DA^{0.5})$		0.580	330.	60.4	15.75	0.0002	0.00007
5	Duration	−10.05	−0.386				0.003	
	$(Cum(DA))^{0.5}$		0.750	10.7	10.7	11.23	0.001	0.0008
6	Duration	−10.04	−0.085				0.20	
	Avg(DA)		0.338	1.40	1.96	7.61	0.002	0.006

Baseline rate: as Log(rate); per day
LRT = likelihood ratio test
Model – rate = $\exp(\alpha + \beta smoker + \gamma sex + \delta(age - 40) + \varepsilon(age - 40)^2) + \theta packyrs + \Phi packyrs^2 + \eta cum(dur) + \mu cum(DA))$ (model 3)
RR = relative rate, at 1 or 2 ppm, or 1 year (duration)
Δ – 2lnL = change in – 2 × ln(likelihood), relative to model 1)

Table 5-14. Company G incidence rate models: exposure metrics (case definition 3: $FEV_1 < LLofN$ and $FEV_1/FVC < LLofN$; n=19)

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	1.13	1.13	—	0.60	—
3	Duration	−9.61	−0.300				0.023	
	Cum(DA)		0.090	2.46	2.46	5.31	0.016	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

Baseline rate: as Log(rate); per day
LRT = likelihood ratio test
Model = rate = $\exp(\alpha + \beta smoker + \gamma sex + \delta(age - 40) + \varepsilon(age - 40)^2) + \theta packyrs + \Phi packyrs^2 + \eta cum(dur) + \mu cum(DA))$ (model 3)
RR = relative rate, at 1 or 2 ppm, or 1 year (duration)
Δ – 2lnL = change in – 2 × ln(likelihood), relative to model 1)

Table 5-15. Company G incidence rate model with duration and cum(DA) (case definition 3: FEV_1 < LLofN and FEV_1/FVC < LLofN; 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
$(Age - 40)^2$	−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

rate = $\exp(\alpha + \beta smoker + \gamma sex + \delta(age - 40) + \varepsilon(age - 40)2) + \theta packyrs + \Phi packyrs^2 + \eta cum(dur) + \mu cum(DA))$
Model likelihood ratio test for cum(DA), LRT = 5.306, *P* = 0.021
t = t-statistic for exposure metric effect

Table 5-16. Company G: observed cases (case definition 1: FEV_1 < LLofN) by duration and cumulative diacetyl

	Observed cases cumulative diacetyl exposure (ppm-yrs)					
Duration (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 definition 1: FEV_1 < LLofN) by duration and cumulative diacetyl

	Person-yrs cumulative diacetyl exposure (ppm-yrs)					
Duration (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

[Akpinar-Elci et al. 2004; CDC 2007; Israel et al. 2009; Kreiss et al. 2002; NIOSH 2006, 2008]. 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 the time of 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 a fixed baseline rate of 0.022 yields a rate ratio that appears to be systematically elevated at < 4 years vs. >= 4 years durations of exposure (employment after 1986) and at high cumulative exposures (Table 5-19). The same situation was observed in the pooled Company K and Company L populations using the first case definition. Out of 25 cases, 20 occur in the < 4 yr duration strata (Table 5-20), with elevated rate ratios predicted for low durations and high exposures (Table 5-21). With the third case definition in the Company G population, the same pattern is observed but now with fewer cases (n=19 vs. 36) and 9 out of 19 in the < 4 yr duration group (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 Poisson regression 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 susceptibility was declining with duration because low-risk individuals are remaining longer in employment, a different Poisson regression model was fit. Using a linear relative rate specification, this model included a term intended to capture excess risk arising from diacetyl exposures (1) in an unknown portion of the population declining with time that has higher susceptibility or (2) due to individual susceptibility declining with duration of exposure. An exponential decline was assumed and halflives of 0.5, 1, and 2 years were evaluated. Using case definition 3, a model

Table 5-18. Company G: baseline rate (case definition 1: FEV_1 < LLofN) 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
Based on Table 5-12, model 3

Table 5-19. Company G: rate ratio (case definition 1: FEV_1 < LLofN) by duration and cumulative diacetyl

	Predicted rate ratio (relative to fixed baseline: 0.022) cumulative diacetyl exposure (ppm-yrs)					
Duration (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
Based on Table 5-13, model 3: predicted rate divided by 0.022 from Table 5-18: dur > 4yrs

Table 5-20. Companies K and L pooled: cases (case definition 1: FEV_1 < LLofN) by duration and cumulative diacetyl

	Observed cases cumulative diacetyl exposure (ppm-yrs)					
Duration (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. Companies K and L pooled: rate ratio (case definition 1: FEV_1 < LLofN) by duration and cumulative diacetyl

	Predicted rate ratio (relative to fixed baseline: 0.004); cumulative diacetyl exposure (ppm-yrs)					
Duration (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
predicted rate divided by 0.004

Table 5-22. Company G: cases (case definition 3: FEV_1 < LLofN and FEV_1/FVC < LLofN) by duration and cumulative diacetyl

	Observed cases cumulative diacetyl exposure (ppm-yrs)					
Duration (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 definition 3: FEV_1 < LLofN and FEV_1/FVC < LLofN) by duration and cumulative diacetyl

	Predicted rate ratio (relative to baseline: 0.0046) cumulative diacetyl exposure (ppm-yrs)					
Duration (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
Based on Table 5-14, model 3: predicted rate divided by 0.0046

with a term of the form $[Avg(DA)]^2 \times exp(-0.69 \times Duration)$, i.e., halflife 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 1–3). Of the choices examined for parameters in the short-duration risk term, the best fit occurred with a halflife 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 (with no diacetyl exposure) relative to a very low baseline rate was 17.7 and, for 1.0 ppm-yr of diacetyl exposure in the "low-risk" group (with duration >4 years and no smoking), the rate ratio was 12.3; the initial high risk (at start of exposure, zero duration, and no smoking) rate ratio at 1 ppm diacetyl was 69.8. A similar result was obtained with case definitions 1 and 2 (data not shown) although, for case definition 1, the exposure parameter estimates were not statistically significant.

The relative fit of various incidence-rate model specifications (case definition 3) 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, models 1–3 vs. 4–6). The best fit was for (a) square root of cumulative exposure with duration term (loglinear relative rate model 5), and for (b) cumulative exposure and the term for a high-risk subpopulation (linear relative rate model 10).

5.3.5 Interpretation of Modeling Results

Multiple linear regression models of continuous spirometry outcomes at Company G reveal that both cum(DA) and $\sqrt{Cum(DA)}$ are the preferred predictors of FEV_1 decline based on model fit. Average exposure was the weakest predictor of $ppFEV_1$. Subsidiary analyses indicate that (1) a dose-rate effect, if present, is small and negative (i.e., effects are not limited to high exposures); (2) bias arising from possible removal of earlier cases was probably small, and (3) the bias introduced by the correction procedure addressing degradation of diacetyl air samples is also small although possibly resulting in underestimation of the diacetyl effect. Evidence for non uniform susceptibility includes the somewhat superior prediction by $\sqrt{Cum(DA)}$ compared to Cum(DA) which may be a reflection of a reduced response in the population at longer durations of exposure.

In the modeling of incidence, fewer cases met the third case definition than the first or second (19 vs. 36, 27) due to the requirement that both $ppFEV_1$ and FEV_1/FVC be less than their LLofN. This was consistent with some restriction as was observed in regression models of FVC (data not shown). Using the third 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 strong association with the term representing short duration of exposure supports the conjecture that the population with "normal" susceptibility was 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 changing population composition with respect to susceptibility 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 risk is unknown and workforce turnover continually introduces a higher-risk segment into employment.

Table 5-24. Incidence rate models using linear relative rate model with term for transient high-risk group (shortdur(DA)) at Company G (case definition 3: FEV_1 < LLofN and FEV_1/FVC < LLofN)

Model no.	Parameter	Estimate	RR	LRT	*P* value
1	−2ln(L) = 353.53				
	intercept	−10.9	Baseline rate = 6.5×10^{-3}		
	smoke_ever	−0.879	0.42		
	Ind:female	1.097	2.30		
	age − 40	0.029	1.03		
	$(age - 40)^2$	−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 = 9.9×10^{-3}		
	smoke_ever	−0.714	0.49		
	Ind:female	0.915	2.50		
	age − 40	0.043	1.04		
	$(age - 40)^2$	−0.002	0.998		
	packyrs	0.155	1.16		
	shortdur(DA)	0.536	1.54	4.50	0.034
3	−2ln(L) = 347.27				
	intercept	−15.5	Baseline rate = 6.8×10^{-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
	shortdur(DA)	79.60	80.6	6.26	0.012
4	−2ln(L) = 345.75				
	intercept	−15.5	Baseline rate = 6.9×10^{-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
	shortdur(DA)	69.82	70.8	7.78	0.0053

LRT = likelihood ratio test

General model:

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

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

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

Models 2,3: shortdur(DA) = $[Avg(DA)]^2\exp(-0.693dur)$, for halflife = 1.0 yr; LRT for exposure terms = 7.97 (2 df)

Model 4: shortdur(DA) = $[Avg(DA)]^2\exp(-0.693dur/2)$, for halflife = 2.0 yr; LRT for exposure terms = 9.52 (2 df)

Table 5-25. Likelihood ratio tests and *P* values for choices of constants defining shortdur(DA) variable at Company G (case definition 3: FEV_1 < LLofN and FEV_1/FVC < LLofN)

	LRT for cum(DA) and shortdur(DA) terms (p)	
	Half-life, b	
	1.0	2.0
Avg(DA)	5.93 (0.052)	6.37 (0.019)
$(Avg(DA))^{2.0}$	7.97 (0.019)	9.52 (0.0086)

LRT = likelihood ratio test, 2df
shortdur(DA) = (Avg Exp) $^{a} \times e^{-0.693dur/b}$

Table 5-26. Relative fit of selected model specifications for incidence rate (case definition 3: FEV_1 < LLofN and FEV_1/FVC < LLofN)

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	$\exp(\alpha + \beta smoker + \gamma sex + \delta(age - 40) + \varepsilon(age - 40)^2) + \theta packyrs + \mu(cum(DA))^{0.5})$	−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	$\exp(\alpha + \beta smoker + \gamma sex + \delta(age - 40) + \varepsilon(age - 40)^2) + \theta packyrs + \eta dur + \mu cum(DA))$	−9.66	348.19
5	$\exp(\alpha + \beta smoker + \gamma sex + \delta(age - 40) + \varepsilon(age - 40)^2) + \theta packyrs + \eta dur + \mu(cum(DA))^{0.5})$	−10.39	344.87
6	$\exp(\alpha + \beta smoker + \gamma sex + \delta(age - 40) + \varepsilon(age - 40)^2) + \theta packyrs + \eta dur + \mu avg(DA))$	−10.64	344.93
	Linear relative rate models (additive exposure terms)		
7	$\{\exp(\alpha + \beta smoker + \gamma sex + \delta(age - 40) + \varepsilon(age - 40)^2))\}\{1 + \theta packyrs + \mu cum(DA)\}$	−10.93	353.53
8	$\{\exp(\alpha + \beta smoker + \gamma sex + \delta(age - 40) + \varepsilon(age - 40)^2))\}\{1 + \theta packyrs + \mu(cum(DA))^{0.5}\}$	−15.36	352.04
9	$\{\exp(\alpha + \beta smoker + \gamma sex + \delta(age - 40) + \varepsilon(age - 40)^2))\}\{1 + \theta packyrs + \mu avg(DA)\}$	−15.37	348.93
10	$\{\exp(\alpha + \beta smoker + \gamma sex + \delta(age - 40) + \varepsilon(age - 40)^2))\}\{1 + \theta packyrs + \mu cum(DA) + \sigma shortdur(DA)\}$	−15.47	345.75
11	$\{\exp(\alpha + \beta smoker + \gamma sex + \delta(age - 40) + \varepsilon(age - 40)^2))\}\{1 + \theta packyrs + \mu(cum(DA))^{0.5} + \sigma shortdur(DA)\}$	−14.46	346.99
12	$\{\exp(\alpha + \beta smoker + \gamma sex + \delta(age - 40) + \varepsilon(age - 40)^2))\}\{1 + \theta packyrs + \mu avg(DA) + \sigma shortdur(DA)\}$	−15.21	346.17

Smaller deviance = better fit
shortdur(DA) ~ $[DA]^2\exp(-0.693dur/2)$ – for half-life = 2.0 yr

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 5.1). With increasing cumulative exposure the FEV_1 distribution would be shifted toward lower values and the segment at immediate risk of falling below the LLofN would be increasing as long as the mean (mode or peak) of the shifted distribution remains above LLofN. This is not what was observed; initially the rate of new cases is generally larger and declines with increasing duration or cumulative exposure (Tables 5-19, 5-21), implying variable susceptibility, i.e., most individuals in the exposed population are losing FEV_1 much faster than those with longer duration of exposure.

5.4 Human Data-based Assessment of Risks

Using the impairment findings from Company G NIOSH employed two approaches for assessing risk of diacetyl exposures. The first was the *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, three risk estimates were derived: for a life-time exposure (45 yr) and also for 2.5- and 10-year exposures

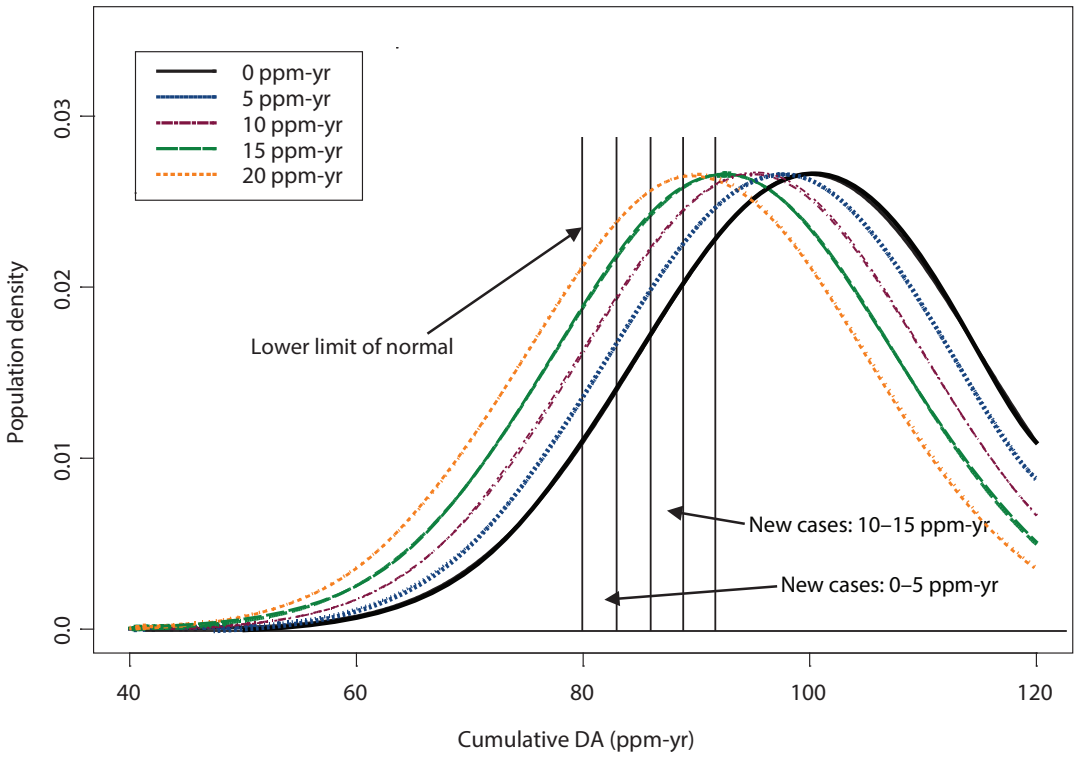


Figure 5-1. New cases expected from a hypothetical population with uniform susceptibility to diminishing percent predicted FEV_1 with increasing cumulative exposure to diacetyl

(more typical employment durations and implying a larger workforce ever exposed). The nominal standard for acceptable risk used was one per thousand excess risk of impairment, a standard choice used in OSHA regulation for chronic diseases.

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 excess 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 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. Unlike animal-based studies where exposures are in discrete levels, the analyses here utilized continuously distributed exposure metrics and a linear statistical model which made unnecessary the point-of-departure procedure commonly used in benchmark dose calculations. This method, however, does require 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 over some time period is called the benchmark dose (BMD).

5.4.1.2 Risk assessment with percent predicted FEV_1 and FEV_1/FVC

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.=−0.50, Table 5-6), the excess prevalence after 2.5, 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, $\sqrt{\text{Cum(DA)}}$, which better predicts $ppFEV_1$ in the full population, substantially lower BMDs result (data not shown); 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. Although $\sqrt{\text{Cum(DA)}}$ better captures the risk of initially employed employees, extrapolation to decreasing durations with this nonlinear metric could introduce considerable error. For this reason NIOSH chose Cum(DA) over $\sqrt{\text{Cum(DA)}}$ as the basis for risk assessment using the BMD procedure. In addition, to address the same issue for early exposures, the BMD was also calculated based on results from the < 4 yr population (Table 5-28) but also for a 45 yr. working lifetime. The resulting excess prevalence estimates were about double those based on the full population.

For impairment defined in relation to LLofN as opposed to some fixed threshold such as the 5th percentile of $ppFEV_1$, the BMD procedure is less direct because LLofN is specific to age, height, gender and race. The distribution of various functions of FEV_1 and LLofN, such as FEV_1/LLofN or (FEV_1– LLofN)/($ppFEV_1$ – LLofN) are not readily specifiable. An alternate approach was taken: in the NHANES population [CDC and NCHS 2011], the cumulative exposure (Cum(DA)) that would reduce an individual's FEV_1 to their LLofN was calculated using the exposure-response estimates from the

Table 5-27. Benchmark dose, based on exposure response with cum(DA) for full population at Company G

	Percent of predicted FEV_1 ($ppFEV_1$)								
				Excess prevalence of impairment (per thousand)					
Diacetyl ppm	Model-predicted $ppFEV_1$			< 60% of predicted	< 5th percentile	< 60% of predicted	< 5th percentile	< 60% of predicted	< 5th percentile
	2.5 yr	10 yr	45 yr	2.5 yr		10 yr		45 yr	
1.0	98.8	95.0	77.5	1.4	8.8	7.4	42.4	126.7	366.8
0.5	99.38	97.5	88.8	0.7	4.3	3.0	18.7	27.9	126.7
0.2	99.75	99.00	95.5	0.3	1.7	1.1	6.9	6.4	37.2
0.1	99.88	99.50	97.8	0.2	0.9	0.5	3.4	2.7	16.6
0.05	99.94	99.75	98.9	0.1	0.4	0.3	1.7	1.2	7.8
0.02	99.98	99.90	99.55	0.1	0.2	0.1	0.7	0.5	3.0
0.01	99.99	99.95	99.78	0.1	0.1	0.0	0.3	0.2	1.5
0.005	99.99	99.98	99.89	0.1	0.1	0.0	0.2	0.1	0.7
0.002	100.0	99.99	99.96	0.0	0.1	0.0	0.1	0.0	0.3
0.001	100.0	100.0	99.98	0.0	0.0	0.0	0.0	0.0	0.1
0.0005	100.0	100.0	99.99	0.0	0.0	0.0	0.0	0.0	0.1
0.0002	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0001	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0

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

preferred regression models of $ppFEV_1$ (coef.=-0.50, -1.07 (< 4 yr); Table 5-6). The prevalence of individuals predicted to be below their LLofN was then calculated in the NHANES III population as a function of exposure over 2.5, 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 for the full population and for < 4 yr (Table 5-29). For FEV_1 below the LLofN (FEV_1) the BMD values were 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 (Table 5-29). In the pooled Company K and Company L population, where reported exposures were lower than at Company G, the estimated 1/1000 BMDs for 45 yr were much lower: for FEV_1, 0.0005 ppm and FEV_1/FVC, 0.0004 ppm (Table 5-30). 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 5-31), and of course, did not depend on

Table 5-28. Benchmark dose, based on exposure response with cum(DA) for duration less than 4 yrs at Company G

Percent of predicted FEV_1 ($ppFEV_1$)									
				Excess prevalence of impairment (per thousand)					
Diacetyl	Model-predicted $ppFEV_1$			< 60% of predicted	< 5th percentile	< 60% of predicted	< 5th percentile	< 60% of predicted	< 5th percentile
ppm	2.5 yr	10 yr	45 yr	2.5 yr		10 yr		45 yr	
1.0	97.3	89.3	51.9	3.3	19.9	25.3	116.5	691.2	872.2
0.5	98.7	94.7	76.0	1.5	9.3	8.1	45.6	148.7	403.3
0.2	99.47	97.9	90.4	0.5	3.6	2.5	15.5	20.9	100.1
0.1	99.73	98.9	95.2	0.3	1.8	1.1	7.3	7.0	39.9
0.05	99.87	99.47	97.6	0.1	0.9	0.5	3.6	2.9	17.7
0.02	99.95	99.79	99.04	0.1	0.3	0.2	1.4	1.0	6.6
0.01	99.97	99.89	99.52	0.0	0.2	0.1	0.7	0.5	3.2
0.005	99.99	99.95	99.76	0.0	0.1	0.1	0.3	0.2	1.6
0.002	99.99	99.98	99.90	0.0	0.0	0.0	0.1	0.1	0.6
0.001	100.0	99.99	99.95	0.0	0.0	0.0	0.1	0.0	0.3
0.0005	100.0	99.99	99.98	0.0	0.0	0.0	0.0	0.0	0.2
0.0002	100.0	100.0	99.99	0.0	0.0	0.0	0.0	0.0	0.1
0.0001	100.0	100.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0

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

duration of work. The 1/1000 BMD for FEV_1, was correspondingly higher: 0.05 ppm diacetyl.

5.4.2 Excess Lifetime Risk for Pulmonary Impairment

5.4.2.1 Methods

Using the life-table approach as implemented in the Biological Effects of Ionizing Radiation 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 in the general population. Although typical applications of the excess lifetime risk calculation are for deaths 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

Table 5-29. Empirical benchmark dose, FEV_1 and FEV_1/FVC based on exposure response with cum(DA) for all employees and for those < 4 yr duration, at Company G

	Empirical BMD Excess prevalence < lower limit of normal (per thousand)											
	FEV_1 all			FEV_1 duration < 4 yr			FEV_1/FVC all			FEV_1/FVC duration < 4 yr		
DA ppm	2.5 yr	10 yr	45 yr	2.5 yr	10 yr	45 yr	2.5 yr	10 yr	45 yr	2.5 yr	10 yr	45 yr
1.0	13.9	68.1	532.5	31.2	188.7	879.4	7.3	30.4	220.5	41.3	287.4	890.8
0.5	6.8	28.9	202.9	14.8	74.5	573.8	3.8	14.1	82.4	19.2	103.0	806.7
0.2	2.6	10.5	58.7	5.9	23.9	161.6	2.4	6.4	27.4	7.5	32.3	243.7
0.1	1.3	5.5	25.7	2.8	11.6	64.1	1.1	3.4	12.1	4.0	15.2	90.1
0.05	0.5	2.6	12.3	1.4	5.9	27.8	0.6	2.4	6.8	2.8	7.5	36.4
0.02	0.2	1.1	4.8	0.4	2.3	10.0	0.4	0.9	3.2	1.1	3.5	13.3
0.01	0.2	0.4	2.5	0.2	1.2	5.3	0.2	0.4	2.1	0.6	2.5	7.0
0.005	0.1	0.2	1.3	0.2	0.4	2.6	0.1	0.4	1.0	0.4	1.1	3.7
0.002	0.1	0.2	0.4	0.1	0.2	1.2	0.1	0.2	0.4	0.2	0.4	2.4
0.001	0.1	0.1	0.2	0.1	0.2	0.4	0.1	0.1	0.3	0.1	0.4	1.0
0.0005	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.2	0.1	0.2	0.6
0.0002	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.4
0.0001	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2

models formed the basis of the calculation (Table 5-24, model 4, and the related model without terms for smoking), 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 65, for a 45-yr exposure. For 2.5 and 10 years of exposure, the life-table exposures start at age 20. Because smoking information was used in modeling, several variants for lifetime risk are presented (Table 5-32). For example, at 0.01 ppm diacetyl, using an incidence rate model (case definition 3) that ignores smoking determinants, the excess lifetime risk (analogous to excess prevalence in the BMD approach) was 3.2/1000. With the model that includes 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 (1) smoking reduces the amount of additional impairment caused by diacetyl over and above that caused by smoking, (2) smoking is a strong competing

Table 5-30. Empirical benchmark dose based on pooled Company K and Company L populations

Diacetyl ppm	Empirical BMD—pooled Company K, L populations excess prevalence (per thousand)					
	FEV_1 < LLofN		FEV_1 < 60 %		FEV_1/FVC < LLofN	
	10 yr	45 yr	10 yr	45 yr	10 yr	45 yr
1.0	854.2	892.5	550.5	994.7	877.5	898.1
0.5	491.4	892.5	108.5	994.4	550.9	898.1
0.2	134.8	823.6	16.7	443.4	135.3	863.9
0.1	54.9	428.0	5.86	84.7	52.0	471.2
0.05	23.6	158.4	2.45	20.4	24.3	160.6
0.02	9.00	48.2	0.88	5.09	9.09	45.6
0.01	4.41	21.2	0.42	2.17	5.29	21.9
0.005	2.29	9.88	0.21	1.00	3.09	10.1
0.002	0.88	4.15	0.08	0.38	1.15	4.50
0.001	0.35	1.94	0.04	0.18	0.71	3.00
0.0005	0.18	1.15	0.02	0.09	0.44	1.32
0.0002	0.09	0.18	0.00	0.03	0.18	0.35
0.0001	0.09	0.35	0.00	0.01	0.18	0.62

Table 5-31. 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
1.0	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

*Method unable to resolve risks below this level

Table 5-32. Excess lifetime risk based on incidence rate model (case definition 3) with term for short duration group at Company G

	Excess lifetime risk (per thousand)								
	2.5 yr			10 yr			45 yr		
Diacetyl ppm	Model a	Model b for non-smokers	Model b for smokers	Model a	Model b for non-smokers	Model b for smokers	Model a	Model b for non-smokers	Model b for smokers
1.0	31.9	110.4	22.5	99.0	320.9	69.5	248.8	659.5	164.6
0.5	15.7	56.0	11.2	51.3	175.7	36.3	140.7	424.0	94.2
0.2	6.2	22.6	4.5	21.0	74.3	14.9	60.8	199.9	41.0
0.1	3.1	11.3	2.2	10.5	37.9	7.5	31.2	105.8	21.1
0.05	1.5	5.7	1.1	5.3	19.1	3.8	15.8	54.5	10.7
0.02	0.6	2.3	0.4	2.1	7.7	1.5	6.4	22.2	4.3
0.01	0.3	1.1	0.2	1.1	3.9	0.8	3.2	11.2	2.2
0.005	0.2	0.6	0.1	0.5	1.9	0.4	1.6	5.6	1.1
0.002	0.1	0.2	0.0	0.2	0.8	0.2	0.6	2.2	0.4
0.001	0.0	0.1	0.0	0.1	0.4	0.1	0.3	1.1	0.2
0.0005	0.0	0.1	0.0	0.1	0.2	0.0	0.2	0.6	0.1
0.0002	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.2	0.0
0.0001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0

case definition 3: FEV_1 < LLof N and FEV_1/FVC < LLof N
Model a: no smoking terms in model of case incidence (Table 5-24, model 4)
Model b: smoking terms in model of case incidence (Table 5-24, model 4) but risk calculated separately for nonsmokers and smokers: 1 pack/day

cause for becoming a case, and, (3) smoking was assumed to continue after age 65.

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, sex, smoking, and *body mass index* – 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 (RRs) 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, and using the exposure response for FEV_1 from the full population and from the < 4 yr group, a life-table analysis produced estimates of excess lifetime risk (Table 5-33) that were comparable (fortuitously) to those based on the incidence of pulmonary impairment, e.g., FEV_1 falling below LLofN (Table 5-32). These estimates of excess mortality are the result of a generic effect of declining FEV_1 on mortality not specific to obliterative

Table 5-33. Excess lifetime risk of mortality due to FEV_1 deficit arising from 45 yrs diacetyl exposure

Diacetyl ppm	FEV_1 effect based on full population	FEV_1 effect based on duration < 4 yr
1.0	221.6	392.1
0.5	121.1	234.1
0.2	51.2	105.0
0.1	26.1	54.6
0.05	13.2	27.8
0.02	5.30	11.3
0.01	2.65	5.66
0.005	1.33	2.84
0.002	0.53	1.14
0.001	0.27	0.57
0.0005	0.13	0.28
0.0002	0.05	0.11
0.0001	0.03	0.06

Based on multiple regression model of fall in percent predicted FEV_1 with diacetyl exposure (0.5% per ppm-yr diacetyl) and on published estimates 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)

bronchiolitis. This generic effect would not adequately predict mortality proceeding from advancing obliterative bronchiolitis disease itself with high exposures to diacetyl.

5.5 Sensitivity Analyses and Alternate Hypotheses

NIOSH conducted sensitivity analyses to the impact of various parameters, variables and assumptions on risk estimates. 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 similar for the different modeling approaches and the diacetyl levels estimated for a given level of lifetime 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 incidence rate of pulmonary impairment, defined three different ways, were compared, the same pattern was observed revealing an unexpected elevation of effect in the low duration group compared to long durations. Exposures in mixers by themselves were shown not to account for the declining respiratory measures in the Company G population, and smoking did not exacerbate the diacetyl effects (actually

was protective). 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 exposure uncertainties prior to the NIOSH surveys, particularly the date when widespread diacetyl exposures commenced 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 because most employees surveyed were hired after 1994.

In constructing the exposure matrix for the plants studied, the decision was made not to apply the humidity correction for air samples below the LOD. To determine if this choice affected the analytical results, analyses were repeated having applied the correction to all air samples. The resulting difference in parameter estimate for the model of percent predicted FEV_1 with the cumulative exposure term was very small: −0.500 vs. −0.499. For the metric, square root of cumulative exposure, the parameter estimate is slightly larger when samples < LOD are corrected: −2.77 (uncorrected) vs. −2.82 (corrected). For the models of FEV_1/FVC there was no change. Therefore there was no impact on risk estimates which were based on these parameter estimates.

Several alternate explanations were considered for the apparent variability in susceptibility:

(1) The proportion of Hispanic employees was higher among the short duration cases: Hispanics also comprised a higher proportion among recent hires and the cross-sectional surveys tended to reflect more recent employees due to high turnover.

(2) Bias from candidate cases lacking styptom onset: using the date of their first qualifying spirometry would tend to increase rather than decrease the estimate of duration of exposure until onset and thus would not account for the short-duration cases.

(3) Recall bias on symptom onset: employees with fast onset probably estimated symptom onset in relation to hire date, which is generally precisely known, not in relation to survey date. For example, an employee with 3 years employment probably would recall that symptoms began after about 6 months on the job, not 2.5 years ago.

(4) Jobs with peak exposures would favor an early onset: this would happen only if the cumulative exposure metric was underestimating the relevant exposure. This could occur with a positive dose-rate effect, but what was observed was, if anything, a negative dose-rate effect (Table 5-5) where summing the square root of air concentrations over time was a much better predictor than summing the square of concentrations. Serious exposure misclassification could cause a pattern indistinguishable from variable susceptibility; employees whose exposures were substantially underestimated would appear to respond more strongly (faster) with adverse health effects and conversely for employees 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, and excluding mixers did not reduce affect estimates. Undoubtedly misclassification was present but a systematic discrepancy in risk by a factor of 10, as observed between the short and long duration groups and others arising from misclassification is 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 diacetyl-exposed employees have proved 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 this potential participation bias.

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, a more credible explanation for the association of impairment with average exposure is the changing composition of the population over 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 duration of observation and 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.

Appropriate 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 employee's risk of impairment which is the clinician's measure of impact, and the risk incurred by the population of employees 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 an employee's FEV_1/FVC is less than 0.7 (or FEV_1 less than or equal to 80%), that would be considered mild COPD [GOLD 2011]. Similarly, if diacetyl exposure decreases the mean pulmonary function of the exposed population by some small increment, this too could be considered an adverse event [ATS 2000].

The health significance of small spirometry changes, such as a 1% decline in FEV_1 after 2 years of exposure at 1 ppm diacetyl, depends partly on whether such changes are early indications of lung pathology that eventually would manifest as obliterative bronchiolitis. In studies of obliterative bronchiolitis arising from lung transplantation, unrelenting irreversible FEV_1 decrements are observed that ultimately lead to the diagnosis of obliterative bronchiolitis and fatal disease [Heng et al. 1998]. However, incomplete knowledge concerning the natural history of obliterative bronchiolitis development with diacetyl exposure is a limitation in the present risk assessment. 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 (about 1% loss) is comparable to changes observed in children, a more vulnerable population, exposed to levels of air pollution that lead to clinical impairment in later life [Gauderman et al. 2004].

Variation in susceptibility poses issues for risk assessment. If less-susceptible individuals are remaining in employment longer, the estimated exposure response for long durations when applied to a hypothetical population of 1,000 employees employed 45 years, will generate excess risk values that understate the true risk of a workforce that turns over more often.

All of the risk assessment procedures used here assume some degree of low-dose linearity, with effects diminishing proportionally with decreasing exposure levels that are held constant over 10 or 45 years. Model linearity was observed particularly after limiting the population to < 4 yr duration. Moreover a significant fraction of career-average exposures fell below 0.01 ppm (17% of employees) a factor of only 2.0 higher than the proposed REL. Thus low-dose extrapolation was limited. 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 5-34). Impairment has been defined here as pulmonary function falling below the lower limit of normal. The BMD estimates for excess prevalence of FEV_1 impairment are within a factor of 2.0 of the life-table estimates of excess lifetime risk (1) using case definitions 2 and 3 (ignoring smoking) and (2) for excess mortality. Excess risk of 1/1,000 corresponds to approximately 0.001–0.005 ppm diacetyl (3.5-17.5 μg/m^3) in the full Company G population. NIOSH has selected Company G risk estimates as 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, determined by NIOSH to be a less adequate basis for risk assessment, the benchmark dose analysis for 1/1,000 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 5-35.

Table 5-34. Risk assessment synthesis: excess prevalence or lifetime risk (per thousand) for 45-yr exposure to diacetyl

Diacetyl ppm	Method							
	BMD—Excess prevalence of impairment (per thousand)				Life-table—Excess lifetime risk (per thousand)			
							All-cause mortality	
	FEV_1 (LLofN)	FEV_1/FVC (LLofN)	FEV_1 (LLofN) < 4 yrs	FEV_1/FVC (LLofN) < 4 yrs	Incidence case defn 2	Incidence case defn 3	All	< 4 yr
0.05	12.3	6.8	27.8	36.4	20.7	15.8	13.2	27.8
0.02	4.8	3.2	10.0	13.3	8.4	6.4	5.3	11.3
0.01	2.5	2.1	5.3	7.0	4.2	3.2	2.7	5.7
0.005	1.3	**1.0**	2.6	3.7	2.1	1.6	1.3	2.8
0.004	**1.1**	0.8	1.9	3.4	1.7	1.3	**1.1**	2.3
0.003	0.6	0.6	1.5	3.0	1.3	**1.0**	0.8	1.7
0.002	0.4	0.4	**1.2**	2.4	**0.9**	0.6	0.5	**1.1**
0.001	0.2	0.3	0.4	**1.0**	0.4	0.3	0.3	0.6

case definition 2: FEV_1/FVC < LLofN

case definition 3: FEV_1 < LLofN and FEV_1/FVC < LLofN

BMD: Based on empirical benchmark dose procedure, 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 1,000 starting with exposure at age 20 through 65, until age 85

1/1000 risk exposures in bold; based on rate model not including smoking determinants.

Table 5-35. Risk assessment synthesis: diacetyl exposure levels (ppm) over 45 yrs predicting excess prevalence or lifetime risk

	Method								
	BMD: Excess prevalence impairment				Life-table: Excess lifetime risk Case onset, definition 3			Life-table: Excess lifetime risk Mortality	
Excess risk	FEV_1 (LLofN)	FEV_1/FVC (LLofN)	FEV_1 (LLofN) < 4 yrs	FEV_1/FVC (LLofN) < 4 yrs	all	for non-smokers	for smokers	all	< 4 yr
1/10	0.30	0.60	0.15	0.11	0.30	0.10	0.50	0.40	0.20
1/100	0.04	0.08	0.02	0.02	0.03	0.01	0.05	0.04	0.02
1/1000	**0.004**	**0.005**	**0.002**	**0.001**	**0.003**	**0.0009**	**0.005**	**0.004**	**0.002**
1/10000	0.0004	0.0005	0.0002	0.0001	0.0002	0.00009	0.0004	0.0004	0.0002
1/100000	0.00004	0.00005	0.00002	0.00001	0.00002	0.000009	0.00004	0.00004	0.00002

case definition 3: 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

< 4 yrs: analyses based on population with < 4 yr exposure to DA, thought to be less affected by healthy employee survivor effect

1/1000 risk exposures in bold.

References

Akpinar-Elci M, Travis WD, Lynch DA, Kreiss K [2004]. Bronchiolitis obliterans syndrome in popcorn production plant employees. Eur Respir J *24*(2):298–302.

ATS (American Thoracic Society) [2000]. What constitutes an adverse health effect of air pollution? Official statement of the American Thoracic Society. Am J Respir Crit Care Med *161*(2 Pt 1):665–673.

Bailer AJ, Stayner LT, Smith RJ, Kuempel ED, Prince MM [1997]. Estimating benchmark concentrations and other noncancer endpoints in epidemiology studies. Risk Anal *17*(6):771–780.

Bang KM, Gergen PJ, Kramer R, Cohen B [1993]. The effect of pulmonary impairment on all-cause mortality in a national cohort. Chest *103*(2):536–540.

CDC (Centers for Disease Control and Prevention) [2007]. Fixed obstructive lung disease among employees in the flavor-manufacturing industry—California, 2004–2007. MMWR *56*(16):389–393.

CDC, NCHS (Centers for Disease Control and Prevention, National Center for Health Statistics) [2011]. National Health and Nutrition Examination Survey. http://www.cdc.gov/nchs/nhanes.htm. Date accessed: March 2011.

Checkoway H, Pearce N, Kriebel D [2004]. Research methods in occupational epidemiology. 2nd ed. New York: Oxford University Press, pp. 101–105.

Clewell HJ, Crump KS [2005]. Quantitative estimates of risk for noncancer endpoints. Risk Anal *25*(2):285–289.

Clewell HJ, Lawrence GA, Calne DB, Crump KS [2003]. Determination of an occupational exposure guideline for manganese using the benchmark method. Risk Anal *23*(5):1031–1046.

Committee on the Biological Effects of Ionizing Radiation [1988]. Health risks of radon and other internally deposited alpha-emitters: biological effects of ionizing radiation (BEIR) IV. Washington, DC: National Academy Press.

Corn M, Esmen NA [1979]. Workplace exposure zones for classification of employee exposures to physical and chemical agents. Am Ind Hyg Assoc J *40*(1):47–57.

Cox-Ganser J, Ganser G, Saito R, Hobbs G, Boylstein R, Hendricks W, Simmons M, Eide M, Kullman G, Piacitelli C [2011]. Correcting diacetyl concentrations from air samples collected with NIOSH Method 2557. J Occup Environ Hyg *8*(2):59–70.

Crump KS [1995]. Calculation of benchmark doses from continuous data. Risk Anal *15*(1):79–89.

Cullen K, Stenhouse NS, Wearne KL, Welborn TA [1983]. Multiple regression analysis of risk factors for cardiovascular disease and cancer mortality in Busselton, Western Australia—13-year study. J Chronic Dis *36*(5):371–377.

Ebi-Kryston KL, Hawthorne VM, Rose G, Shipley MJ, Gillis CR, Hole DJ, Carmen W, Eshleman S, Higgins MW [1989]. Breathlessness, chronic bronchitis and reduced pulmonary function as predictors of cardiovascular disease mortality among men in England, Scotland and the United States. Int J Epidemiol *18*(1):84–88.

Ferrer M, Villasante C, Alonso J, Sobradillo V, Gabriel R, Vilagut G, Masa JF, Viejo JL, Jimenez-Ruiz CA, Miravitlles M [2002]. Interpretation of quality of life scores from the St George's Respiratory Questionnaire. Eur Respir J *19*(3):405–413.

Ferris BG [1978]. Epidemiology standardization project (American Thoracic Society). Am Rev Respir Dis *118*(6 Pt 2):1–120.

Gauderman WJ, Avol E, Gilliland F, Vora H, Thomas D, Berhane K, McConnell R, Kuenzli N, Lurmann F, Rappaport E, Margolis H, Bates D, Peters J [2004]. The effect of air pollution on lung development from 10 to 18 years of age. N Engl J Med *351*(11):1057–1067.

GOLD (Global Initiative for Chronic Obstructive Lung Disease) [2011]. COPD diagnosis and management at-a-glance desk reference. http://www.goldcopd.org/uploads/users/files/GOLD_AtAGlance_2010.pdf. Date accessed: August 2011.

Hankinson JL, Odencrantz JR, Fedan KB [1999]. Spirometric reference values from a sample of the general U.S. population. Am J Respir Crit Care Med *159*(1):179–187.

Heng D, Sharples LD, McNeil K, Stewart S, Wreghitt T, Wallwork J [1998]. Bronchiolitis obliterans syndrome: incidence, natural history, prognosis, and risk factors. J Heart Lung Transplant *17*(12):1255–1263.

Hole DJ, Watt GC, Davey-Smith G, Hart CL, Gillis CR, Hawthorne VM [1996]. Impaired lung function and mortality risk in men and women: findings from the Renfrew and Paisley prospective population study. BMJ *313*(7059):711–715; discussion 715–716.

Israel L, Kim T, Prudhomme J, Bailey R, Harber P [2009]. Workplace spirometry: early detection benefits individuals, employee groups and employers. http://www.thoracic.org/clinical/ats-clinical-cases/pages/workplace-spirometry-early-detection-benefits-individuals,-employee-groups-and-employers.php. Date accessed: September 2010.

Knuiman MW, James AL, Divitini ML, Ryan G, Bartholomew HC, Musk AW [1999]. Lung function, respiratory symptoms, and mortality: results from the Busselton Health Study. Ann Epidemiol *9*(5):297–306.

Kreiss K [2007]. Flavoring-related bronchiolitis obliterans. Curr Opin Allergy Clin Immunol *7*(2):162–167.

Kreiss K, Gomaa A, Kullman G, Fedan K, Simoes EJ, Enright PL [2002]. Clinical bronchiolitis obliterans in employees at a microwave-popcorn plant. N Engl J Med *347*(5):330–338.

Kuller LH, Ockene JK, Townsend M, Browner W, Meilahn E, Wentworth DN [1989]. The epidemiology of pulmonary function and COPD mortality in the multiple risk factor intervention trial. Am Rev Respir Dis *140*(3 Pt 2):S76–81.

Kullman G, Boylstein R, Jones W, Piacitelli C, Pendergrass S, Kreiss K [2005]. Characterization of respiratory exposures at a microwave popcorn plant with cases of bronchiolitis obliterans. J Occup Environ Hyg *2*(3):169–178.

Lockey JE, Hilbert TJ, Levin LP, Ryan PH, White KL, Borton EK, Rice CH, McKay RT, LeMasters GK [2009]. Airway obstruction related to diacetyl exposure at microwave popcorn production facilities. Eur Respir J *34*(1):63–71.

Mannino DM, Davis KJ [2006]. Lung function decline and outcomes in an elderly population. Thorax *61*(6):472–477.

Mannino DM, Reichert MM, Davis KJ [2006]. Lung function decline and outcomes in an adult population. Am J Respir Crit Care Med *173*(9):985–990.

National Research Council [2009]. Science and decisions: advancing risk assessment. Washington, DC: National Academies Press.

National Toxicology Program [2013a]. Testing Status of 2,3-Butanedione (Diacetyl). http://ntp.niehs.nih.gov/?objectid=BDAAC16E-123F-7908-7B81117E04E12A40. Date accessed: August 2013.

National Toxicology Program [2013b]. Testing Status of Acetoin. http://ntp.niehs.nih.gov/?objectid=BDC958F4-123F-7908-7B566135E40DAE85. Date accessed: August 2013.

NIOSH [2003a]. Hazard evaluation and technical assistance report: Agrilink Foods Popcorn Plant, Ridgway, IL. By Sahakian N, Choe K, Boylstein R, Schleiff P. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2002–0408–2915.

NIOSH [2003b]. Hazard evaluation and technical assistance report: B.K. Heuermann Popcorn, Inc, Phillips, NE. By Kanwal R, Martin S. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2001–0517.

NIOSH [2003c]. Hazard evaluation and technical assistance report: Nebraska Popcorn, Clearwater, NE. By Kanwal R. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2002–0089.

NIOSH [2004a]. Hazard evaluation and technical assistance report: American Pop Corn Company, Sioux City, IA. By Kanwal R, Boylstein R, Piacitelli C. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2001–0474–2943.

NIOSH [2004b]. Hazard evaluation and technical assistance report: ConAgra Snack Foods, Marion,

OH. By Kanwal R, Kullman G. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2003–0112–2949.

NIOSH [2006]. Hazard evaluation and technical assistance report: Gilster-Mary Lee Corporation, Jasper, MO. By Kanwal R, Kullman G, Fedan K, Kreiss K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2000–0401–2991.

NIOSH [2008]. Hazard evaluation and technical assistance report: Gold Coast Ingredients, Inc., Commerce, CA. By Bailey R, McKernan L, Dunn K, Sahakian N, Kreiss K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2007–0033–3074.

Park RM, Bowler RM, Eggerth DE, Diamond E, Spencer KJ, Smith D, Gwiazda R [2006]. Issues in neurological risk assessment for occupational exposures: the Bay Bridge welders. Neurotoxicology *27*(3):373–384.

Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF, Wanger J [2005]. Interpretative strategies for lung function tests. Eur Respir J *26*(5):948–968.

Rodriguez BL, Masaki K, Burchfiel C, Curb JD, Fong KO, Chyou PH, Marcus EB [1994]. Pulmonary function decline and 17-year total mortality: the Honolulu Heart Program. Am J Epidemiol *140*(5):398–408.

Ryan G, Knuiman MW, Divitini ML, James A, Musk AW, Bartholomew HC [1999]. Decline in lung function and mortality: the Busselton Health Study. J Epidemiol Community Health *53*(4):230–234.

Sabia S, Shipley M, Elbaz A, Marmot M, Kivimaki M, Kauffmann F, Singh-Manoux A [2010]. Why does lung function predict mortality? Results from the Whitehall II Cohort Study. Am J Epidemiol *172*(12):1415–1423.

SAS Institute Inc. [2008]. SAS® 9.2 Enhanced Logging Facilities. Cary, NC: SAS Institute Inc.

Schroeder EB, Welch VL, Couper D, Nieto FJ, Liao D, Rosamond WD, Heiss G [2003]. Lung function and incident coronary heart disease: the atherosclerosis risk in communities study. Am J Epidemiol *158*(12):1171–1181.

Schunemann HJ, Dorn J, Grant BJ, Winkelstein, W Jr., Trevisan M [2000]. Pulmonary function is a long-term predictor of mortality in the general population: 29-year follow-up of the Buffalo Health Study. Chest *118*(3):656–664.

Sin DD, Wu L, Man SF [2005]. The relationship between reduced lung function and cardiovascular mortality: a population-based study and a systematic review of the literature. Chest *127*(6):1952–1959.

Smith TJ [1992]. Occupational exposure and dose over time: limitations of cumulative exposure. Am J Ind Med *21*(1):35–51.

SSA (Social Security Administration) [2005]. Life table for the United States Social Security Area 1900-2100: actuarial study #120. Table 6. http://www.ssa.gov/OACT/NOTES/as120/LifeTables_Tbl_6_2000.html. Date accessed: May 2010.

Wise RA [2006]. The value of forced expiratory volume in 1 second decline in the assessment of chronic obstructive pulmonary disease progression. Am J Med *119*(10 Suppl 1):4–11.

This page intentionally left blank.

6 Quantitative Risk Assessment Based on Animal Data

6.1 Introduction

6.1.1 Diacetyl

Dose-response data for diacetyl toxicity in laboratory animals are available, and there are limited but useful animal data on the toxicity of 2,3-pentanedione. Although the NIOSH REL for diacetyl is based on the analysis of human data described in Chapter 5, NIOSH has assessed the animal data for diacetyl to determine whether they are consistent with the human data. For 2,3-pentanedione, NIOSH has conducted a comparative potency analysis, comparing the toxicity of inhaled 2,3-pentanedione to that of diacetyl. These quantitative risk assessments are described below. NIOSH interpretation of the findings and implications for occupational exposure recommendations for diacetyl are described below and in Chapter 7.

Laboratory 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. [2008] reported similar adverse respiratory effects in mice exposed by inhalation to diacetyl for up to 12 weeks. Adverse nasal and lung effects were observed with the latter found in the bronchial, peribronchial, and peribronchiolar regions.

The NTP has issued findings from a 90-day inhalation study of diacetyl in both mice and rats [National Toxicology Program 2011]. Adverse effects were observed in the nose, larynx, trachea, and bronchi in mice and rats. Because the 2011 NTP study had the longest exposure durations among all experimental animal studies, included two species, and used more animals per dose group than the Morgan et al.[2008] study, it was used in the dose-response analysis to BMDs, the lower bound on the BMDs (BMDLs), and corresponding human equivalent concentrations (HECs), as discussed below.

6.1.2 2,3-Pentanedione

Histopathological data from repeated-exposure inhalation toxicology studies with 2,3-pentanedione were first published in 2012, but are limited to 2-week exposures using small numbers of animals [Morgan et al. 2012]. Although these data are limited, it is possible to compare the toxicity produced by 2,3-pentanedione to that produced by diacetyl under similar conditions, and thus estimate the potency of 2,3-pentanedione relative 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 an estimate of the toxic potency of 2,3-pentanedione relative to that of diacetyl. Like diacetyl, 2,3-pentanedione is a reactive alpha-dicarbonyl compound that can damage protein [Epperly and Dekker 1989; Morgan et al. 2016]. In acute inhalation studies, 2,3-pentanedione has respiratory epithelial toxicity comparable to diacetyl [Hubbs et al. 2012]. Recently, bronchial fibrosis has been documented in rats inhaling either 2,3-pentanedione or diacetyl for 2 weeks [Morgan et al., 2016].

6.2 Methods

6.2.1 Data

6.2.1.1 Diacetyl

The response data that were analyzed were obtained from the experimental study reported by the NTP [2011]. Male and female Wistar-Han rats and male and female B6C3F$_1$ hybrid mice were exposed to diacetyl vapors at concentrations of 6.25, 12.5, 25, 60, and 100 ppm, 6 hours per day, 5 days per week, for 13 weeks. The microscopic evaluations of tissues from the larynx, lung, nose, and trachea described whether or not one or more lesions were detected, the types of lesions that were detected, and the assignment of a numeric score describing the lesion's severity on an ordinal scale (1-minimal, 2-mild, 3-moderate, 4-marked) for each type that was detected. Descriptions of the types of lesions observed among rats and mice that were considered for this analysis are given in Tables 6-1 and 6-2, respectively.

6.2.1.1 2,3-Pentanedione

The results of a 2-week inhalation study of 2,3-pentanedione toxicity were reported by Morgan et al. [2012]. Individual animal data

Table 6-1. Respiratory system lesions observed in rats exposed to diacetyl that were considered for this analysis

Tissue	Response
Larynx	Inflammation, Chronic Active
Larynx	Epithelium, Necrosis
Larynx	Respiratory Epithelium, Hyperplasia
Larynx	Respiratory Epithelium, Metaplasia, Squamous
Larynx	Respiratory Epithelium, Regeneration (Females only)
Larynx	Squamous Epithelium, Hyperplasia*
Lung	Infiltration Cellular, Histiocyte
Lung	Inflammation, Eosinophil or Acute
Lung	Bronchiole, Epithelium, Hyperplasia
Lung	Bronchus, Inflammation, Chronic (Males only)
Lung	Bronchus, Epithelium, Hyperplasia†
Lung	Bronchus, Epithelium, Necrosis
Lung	Bronchus, Epithelium, Regeneration
Nose	Inflammation, Suppurative
Nose	Lymphoid Tissue, Hyperplasia
Nose	Olfactory Epithelium, Atrophy
Nose	Olfactory Epithelium, Degeneration
Nose	Olfactory Epithelium, Metaplasia, Respiratory
Nose	Olfactory Epithelium, Necrosis
Nose	Respiratory Epithelium, Hyperplasia
Nose	Respiratory Epithelium, Metaplasia, Squamous
Nose	Respiratory Epithelium, Necrosis
Nose	Turbinate, Atrophy
Trachea	Inflammation, Chronic Active
Trachea	Epithelium, Regeneration
Trachea	Epithelium, Hyperplasia
Trachea	Epithelium, Metaplasia, Squamous
Trachea	Epithelium, Necrosis

*Includes two males classified as having mild "Squamous Epithelium, Hyperplasia, Atypical"

†Includes three males and four females classified as having mild "Bronchus, Epithelium, Hyperplasia, Atypical"

Table 6-2. Respiratory system lesions observed in mice exposed to diacetyl that were considered for this analysis

Tissue	Response	Tissue	Response
Larynx	Inflammation, Chronic Active	Nose	Olfactory Epithelium, Atrophy
Larynx	Epithelium, Necrosis	Nose	Olfactory Epithelium, Metaplasia, Respiratory
Larynx	Respiratory Epithelium, Hyperplasia	Nose	Respiratory Epithelium, Metaplasia, Squamous
Larynx	Respiratory Epithelium, Metaplasia, Squamous*	Nose	Respiratory Epithelium, Necrosis
Larynx	Respiratory Epithelium, Regeneration	Nose	Respiratory Epithelium, Regeneration¶
Larynx	Squamous Epithelium, Hyperplasia†	Nose	Turbinate, Atrophy
Lung	Bronchus, Inflammation, Chronic	Trachea	Inflammation, Chronic Active
Lung	Bronchus, Epithelium, Hyperplasia‡	Trachea	Epithelium, Degeneration or Regeneration**
Lung	Bronchus, Epithelium, Regeneration§	Trachea	Epithelium, Hyperplasia
Nose	Inflammation, Suppurative	Trachea	Epithelium, Metaplasia, Atypical Squamous

*Includes lesions classified as "Respiratory Epithelium, Metaplasia, Atypical Squamous"

†Includes lesions classified as "Squamous Epithelium, Hyperplasia, Atypical"

‡Includes lesions classified as "Bronchus, Epithelium, Hyperplasia, Atypical"

§One male classified as having a minimal "Bronchus, Epithelium, Degeneration" lesion was pooled with 10 other males having a regenerative response.

¶One male and two females classified as having a "Respiratory Epithelium, Degeneration" lesion were pooled with 20 other males, and 20 other females having the regenerative response.

**Seven males and seven females had only the regenerative response, and 12 males and 11 females had only the degenerative response.

from this study were graciously provided for this analysis by Dr. Daniel Morgan, National Institute for Environmental Health and Safety (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 B6C3F1 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 tissue and pathological endpoints that could be modeled successfully for both 2,3-pentanedione and diacetyl (for comparative purposes) are listed in Table 6-3.

In addition to the 13-week NTP bioassay data described above for diacetyl, the 2,3-pentanedione data were also compared to data for diacetyl from Morgan et al. [2008]. These data describe the pathological responses of male 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. [2008] publication, tables of individual

Table 6-3. Pathological endpoints associated with exposure to 2,3-pentanedione that were modeled in this analysis

Tissue	Description of response
Lung	Bronchus, Inflammation, Chronic
Lung	Bronchus, Epithelium, Regeneration
Nose	Inflammation, Suppurative
Nose	Olfactory Epithelium, Atrophy
Nose	Respiratory Epithelium, Metaplasia
Nose	Respiratory Epithelium, Necrosis
Nose	Respiratory Epithelium, Regeneration

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 Analytical approach

An empirical approach based on parametric regression modeling of the ordinal response data was adopted to maximize the information available for analysis from the limited numbers of rodents* in order to assess the potency of diacetyl to increase risk and to assess the relative potency of the two chemicals.

6.2.2.1 Benchmark concentration analysis for rats exposed to diacetyl

The assessment of the potency of diacetyl to increase risk employed the benchmark dose approach that was originally proposed for risk assessment of non-cancer responses by Crump [1984]. It provides a general framework that accommodates a range of responses including responses observed on dichotomous†, ordinal, and continuous scales. It has received extensive development over the past three decades, and it has become an accepted approach for risk assessment [EPA 2012]. Benchmark concentration (BMC) estimates for the pathological endpoints listed in Table 6-1 (for rats) were based on modeling of the exposure concentrations and the associated pathology. In order to avoid the loss of information inherent in dichotomizing ordinal response data, a categorical regression procedure for ordinal data was used to estimate benchmark concentrations. Categorical regression has been previously used in the analysis of toxicological data with multiple levels of severity [Guth et al. 1997; Haber et al. 2001]. The severity scores‡ for each tissue and type of lesion were assumed to be samples from a multinomial distribution following a complementary§ cumulative logistic model fitted separately for each species and sex as follows:

$$logit(\Pr(Y_{ci} \geq j)) =$$

$$\log\left(\frac{Pr(Y_{ci} \geq j)}{1 - Pr(Y_{ci} \geq j)}\right) = \alpha_j + \beta \cdot conc_{ci}$$

where

Y_{ci} denotes the corresponding severity score of the i^{th} rodent exposed to concentration, $conc_c$,

$j \in$ element of {*observed severity scores excluding zero*} for the corresponding tissue and type of lesion,

$\Pr(Y_{ci} \geq j)$ denotes the expected proportion of response score Y_{ci} greater than or equal to j, each α_j is an unknown real-valued parameter with $\alpha_{j'} < \alpha_j$ for $j' > j$, and β is an unknown real-valued parameter describing the slope of the effect of concentration on the logit scale.

*$5 \leq n \leq 10$ rodents were used per species-sex-exposure group.

†Dichotomous responses are often referred to as quantal responses.

‡When no evidence of the lesion being modeled was detected a severity score of zero (0) was assigned.

§The term complementary discerns this model from an equivalent cumulative logistic model of $Pr(Y_{ci} \geq j)$.

The logistic model is based on the logit transformation above which maps the range of expected response proportions, $0<p<1$, to $(-\infty, \infty)$; hence, models defined in terms of the transform constrain the expected proportions to the appropriate range. It is readily parameterized so that this form of the systematic relation applies under varying conditions that are consistent with biological considerations including the redefinition of the response categories by merging them [McCullagh 1980]; this specifically includes merging them to form the dichotomous responses more familiar to toxicology while preserving the *interpretations* of the model parameters thereby facilitating its application. The method of maximum likelihood was applied in order to fit[¶] the model, and a likelihood ratio (*LR*) test for a (non-null) dose-response was performed. Adequacy of the fit was assessed by performing two statistical tests, i.e., a score test for separate slopes (a slope for each unique value of *j*) and a *LR* test for an unrestricted multinomial distribution. The null distribution of the statistic of each test was approximated by its asymptotic chi-square distribution. For those models having a significant dose-response ($P<0.05$) and an adequate fit ($P>0.05$) on both tests, BMCs were estimated corresponding to the concentrations that increased expected proportions by 0.10 over controls[**] for severity scores of 1+ (lesion was at least minimal) and 2+ (lesion exceeded minimal severity). Ninety-five percent confidence intervals for the BMC were calculated from percentiles of 200,000 samples of the asymptotic multivariate normal distribution of the MLE of the model parameters[††]; both a two-sided 95% confidence interval and a lower one-sided 95% confidence limit (BMCL) were estimated.

[¶]The Logistic procedure of SAS™ 9.3 was used.

[**](i.e., a benchmark response of 0.10 for "added risk")

[††]The function, rmvnorm, of Splus with mean=MLE and covariance matrix=estimate of Cov(MLE) was used.

6.2.2.2 Benchmark concentration analysis for mice exposed to diacetyl

Benchmark concentration estimates for the pathological endpoints listed in Table 6-2 (for mice) were developed as described above for the rat data; however, an analysis of the residual errors of the fitted models provided substantial evidence against the model for the data on mice (Figure 6-1).

These residuals have mean equal to zero asymptotically if the linear-in-concentration model is correct. However, the distribution of the residuals of Figure 6-1 is shifted above zero at 50 ppm corresponding to underprediction and the distribution is shifted below zero corresponding to overprediction at 100 ppm. Figure 6-1 provides support for making a modification of the dose-response model in a manner that allows for a reduction of the rate of increase of the response at high doses. Because mice are able to substantially alter their breathing rates in a dose-dependent manner when exposed [Larsen et al. 2009; Morgan et al. 2008] the model of the data for mice was modified to include a quadratic dose term to allow it to more closely fit the data in the high-dose region of the dose-response relationship; this term was parameterized to represent a directly proportional relationship of the change in breathing rate with concentration relative to the breathing rate of the controls. The resulting estimate for male mice exposed to diacetyl was compared with corresponding ventilation measurements provided by Dr. Daniel Morgan, NIEHS (personal communication to Randall Smith, NIOSH, June 5, 2014). In addition, two parameters allowing for adjustment of the intercepts of each sex and a third parameter allowing for adjustment of the effect of exposure were added to the model to account for the varying durations of these studies. This model was further

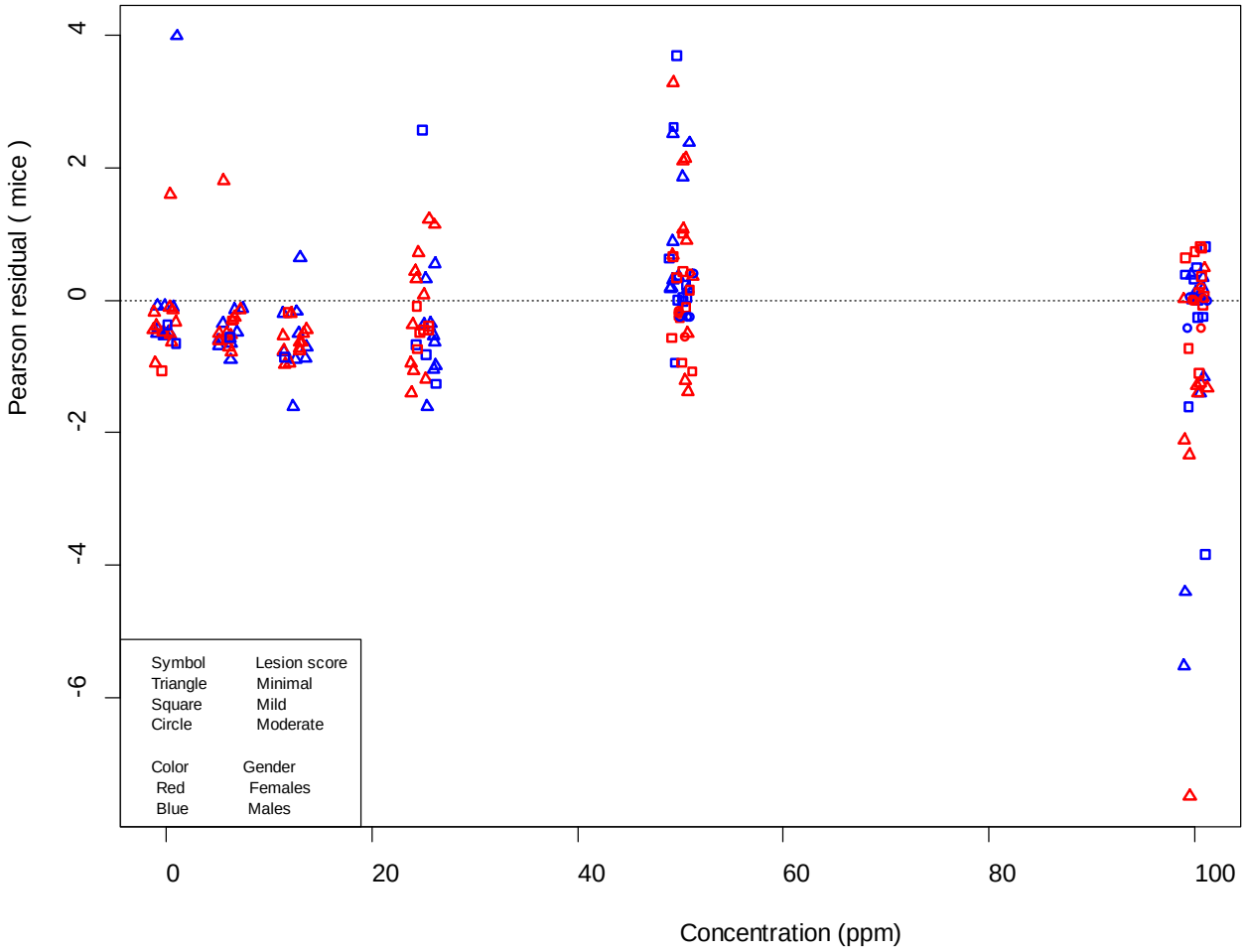


Figure 6-1. Pearson residuals of complementary cumulative logistic models with linear effect of concentration fitted to data on mice. The points have been slightly jittered horizontally to improve resolution.

extended to incorporate the comparative potency analysis of 2,3-pentanedione relative to diacetyl and incorporated an allowance for the responses of each mouse to be correlated by including random effects. It is described below in section 6.2.2.7.

6.2.2.3 Extrapolation of rodent benchmark concentrations to humans

Extrapolation of rodent BMCs to humans was based on a PBPK/CFD model for diacetyl [Gloede et al. 2011; Morris and Hubbs 2009]. The Gloede et al. [2011] extension of the Morris and Hubbs [2009] model predicts tissue concentrations of diacetyl for mucosal surfaces in the nose, trachea, bronchi, and bronchioles of rats and humans exposed to 1 ppm diacetyl. Nose-breathing and mouth-breathing humans are considered, as well as the effects of light exercise as might be expected to occur in the workplace. The Gloede et al. [2011] model assumes mouth breathing during light

exercise conditions. For extrapolation purposes, an 8-hour work day was considered to consist of 2.5 hours of sedentary exposure and 5.5 hours of light exercise, as described by the International Commission on Radiological Protection (ICRP) human respiratory tract model [ICRP 1994]. The ICRP model assumes 20 breaths per minute and a tidal volume of 1,250 mL for light exercise and 12 breaths per minute and a tidal volume of 625 mL for sedentary sitting, for a total inhalation volume of 9.6 m^3 in an 8-hour work day. Therefore, to extrapolate from rodents to humans, the BMC estimates described above were adjusted by a weighted average of the rat:human ratios of the predicted tissue concentrations for a particular anatomical region, under sedentary and light exercise conditions. The Gloede et al. [2011] estimates incorporating tissue metabolism (V_{max} for the rat, and K_{cat} for humans) were used, because local metabolism is predicted to impact significantly on the local tissue concentration [Gloede et al. 2011] (Table 3). For example, the predicted tissue diacetyl concentration for the proximal tracheal mucosa of a rat exposed to 1 ppm diacetyl is 0.33 μM, while the predicted tissue concentration for the same anatomical region is 1.4 μM in a sedentary nose-breathing human and 2.5 μM in a mouth-breathing exercising human. The rat BMCs based on pathological changes to this anatomical region were divided by a factor of (1.4 μM * 2.5 hours + 2.5 μM * 5.5 hours)/(0.33 μM * 6 hours), or 8.71. The factor of 6 hours in the denominator adjusts for the 6-hour/day duration of the experimental exposures, as compared to the 8-hour workday assumed for occupational exposures. Gloede et al. [2011] did not report tissue concentration estimates for the larynx; BMC extrapolation for this region was based on the tissue concentrations estimated for the proximal trachea. Gloede et al. [2011] reported tissue concentrations for both mainstem and small bronchi, and BMC extrapolation for bronchial endpoints were based on the mean of the rat:human ratios of tissue concentrations for mainstem bronchi and small bronchi. Rat to human scaling for the alveoli was based on the estimated fractional penetration of diacetyl through the bronchioles in the Gloede et al. PBPK model, provided by Dr. John Morris, University of Connecticut (personal communication to Dr. David A. Dankovic, NIOSH, November 8, 2012). The rat:human extrapolation factors used are shown in Table 6-4.

6.2.2.4 Extrapolation of BMCs and BMCLs from the mouse to the rat

Because no PBPK model for diacetyl exposures in the mouse is currently available, the rat PBPK model [Gloede et al. 2011] was extended to the mouse using the EPA reference concentration (RfC) methodology [EPA 1994]. In the RfC methodology, the deposition and uptake of volatile chemicals are estimated from a combination of chemical characteristics (i.e., reactivity and solubility) and the physiological characteristics of the relevant species (i.e., minute ventilation and the surface area of the relevant portion of the respiratory tract). Diacetyl is classified as a "category 1" gas in the RfC methodology because of its high water solubility. Category 1 gases are not expected to reach the pulmonary region in high concentration, but rather to be deposited primarily in the upper respiratory tract and the tracheobronchial region. This is consistent with the behavior of diacetyl in the Gloede et al. [Gloede et al. 2011] PBPK model, so that the classification of diacetyl as a category 1 gas appears to be appropriate.

Interspecies dosimetric adjustments via the RfC methodology are based on an estimate of the regional gas dose ratio (RGDR). The RGDR estimates the ratio of gas deposition with a given respiratory tract region in the two species being compared.

Table 6-4. Factors for rodent-to-human extrapolation of airway tissue concentrations of diacetyl, based on Gloede et al. [2011]

Species	Human			Human (light work)	Human (light work)
Breathing via rest/exercise	nose rest	mouth rest	mouth exercise	nose + mouth rest + exercise*	nose + mouth rest + exercise*
	Human-to-rat ratio†				Human-to-mouse ratio‡
Proximal nose	1.6	0	0	0.67	0.3
Proximal trachea	4.2	6.1	7.6	8.7	2.7
Mainstem bronchi	10.0	14.0	21.0	23.0	7.3
Small bronchi	7.2	10.0	32.2	32.0	10.0
Average bronchi§	8.6	12.0	26.6	28.0	8.7
Bronchioles	5.0	7.3	40.9	40.0	12.0
Alveoli¶	4.69	—	15.0	15.7	4.9

"Light work" was estimated to be a combination of 2.5 hours at rest, with nasal breathing, plus 5.5 hours of exercise, with mouth breathing, per 8-hour work day; this was compared to a 6-hour/day exposure for rodents in the experimental studies.

†Rat-to-human scaling based on the overall catalytic rate, K_{cat}, in Gloede et al. [2011]Table 3, except as noted below for alveoli.

‡Mouse-to-human scaling assuming mouse is 2.4 times as sensitive as the rat for nasal effects and 3.2 times as sensitive for tracheobronchial effects, based on the regional gas dose ratio (see section 6.2.2.4)

§"Average bronchi" = arithmetic mean of values for mainstem and small bronchi

¶Rat to human scaling for the alveoli was based on the estimated fractional penetration of diacetyl through the bronchioles in the Gloede et al. PBPK model.

For the ET region, the RGDR is calculated [EPA 1994], eqn. 4-18, as:

$$RGDR_{ET} = \frac{Dose_{ET_A}}{Dose_{ET_B}} \approx \frac{\left(\frac{V_E}{SA_{ET}}\right)_A}{\left(\frac{V_E}{SA_{ET}}\right)_B}$$

where:

V_E = minute volume (mL/min = cm^3/min)
SA = surface area (cm^2)
ET = a subscript denoting the extrathoracic region
A, B = subscripts denoting experimental animal and target species, respectively

For the TB region, the RGDR is calculated [EPA 1994], eqn. 4-22, as:

$$RGDR_{TB} = \frac{Dose_{TB_A}}{Dose_{TB_B}} = \frac{\left(\frac{V_E}{SA_{TB}}\right)_A}{\left(\frac{V_E}{SA_{TB}}\right)_B} \cdot \frac{\left(e^{-\left(\frac{SA_{ET}}{V_E}\right)}\right)_A}{\left(e^{-\left(\frac{SA_{ET}}{V_E}\right)}\right)_B}$$

where:

V_E = minute volume (mL/min = cm^3/min)
SA = surface area (cm^2)
TB = a subscript denoting the tracheobronchial region
ET = a subscript denoting the extrathoracic region
A, B = subscripts denoting experimental animal and target species, respectively

The values assumed for V_E and SA, and the resulting RGDR values for mouse-to-rat extrapolation, are shown in Table 6-5. The rat V_E value is based on data from Gloede et al. [2011], and the mouse V_E was taken from Morgan et al. [2008]. The SA values are from EPA [1994].

The RGDR is used to adjust a point of departure (POD), i.e., a BMC or BMCL in the laboratory species to an equivalent concentration in the target species as follows:

$POD_{BEC} = POD_A * RGDR$

where:

POD_{BEC} = POD equivalent concentration in the target species;

POD_A = POD in the experimental species; and

RGDR = Species A-to-species B regional gas dose ratio for the appropriate region of the respiratory tract.

Although the RGDR is typically used to develop human equivalent concentrations from experimental animal data, in this case it is used to develop a rat equivalent concentration for a point of departure estimated from experimental data in the mouse. The Gloede et al. [2011] PBPK model is then used to extrapolate from the rat equivalent concentration to a human equivalent concentration.

6.2.2.5 Duration adjustment and final human equivalent concentration conversions

Adjustment for the daily duration of exposure (6 hours/day for the NTP experimental study vs. 8 hours/day assumed for occupational exposures) is included in the PBPK model-based extrapolation from rodents to humans, as described in section 6.2.2.2 above; therefore, no additional adjustment for exposure hours per day is needed. The experimental exposure protocol of five exposures per week matches the assumed occupational exposure pattern, so that no adjustment for days exposed per week is required in extrapolating from animals to humans. Occupational exposures may take place for an entire working lifetime, which is assumed to be up to 45 years in duration. Ideally, the datasets used for quantitative risk assessment of occupational exposures to toxicants would include data from 2-year rodent bioassays; however, in this case the available data are limited to exposures of 13 weeks or less. An 8-fold dosimetric adjustment (104 weeks/13 weeks) could be considered in order to account for this discrepancy; however, this appears to be unnecessary for diacetyl.

Table 6-5. Calculation of RGDR for mouse-to-rat extrapolation

Species	V_E* (mL/min)	URT SA† (cm^2)	TB SA‡ (cm^2)	URT RGDR§	TB RGDR¶
Rat	264.0	15	22.5	—	—
Mouse	128.5	3	3.5	2.4	3.2

*Minute volume ventilation

†Upper respiratory tract surface area

‡Tracheobronchial surface area

§Mouse-to-rat regional gas dose ratio for the upper respiratory tract

¶Mouse-to-rat regional gas dose ratio for the tracheobronchial region

This conclusion is based on the analysis of Allen [2009a], who concluded that the 6- and 12-week mouse 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 small number of animals used in this study, the power to detect differences between the 6-week and 12-week experiments is limited. As a consequence of the limited duration of the experimental studies and the limited ability to detect differences between the responses at 6 and 12 weeks, the possibility of increased toxicity with lifetime exposure cannot be entirely ruled out. This possibility was addressed through the application of an uncertainty factor (UF) – discussed below – rather than a dosimetric adjustment.

6.2.2.6 Application of uncertainty factors

The HECs are estimates of frankly toxic exposure levels, and must be adjusted by the application of UFs to allow for uncertainty in animal-to-human extrapolation, interindividual variability, and less than lifetime exposure. In general, these UFs are assumed to be 10-fold for animal-to-human extrapolation and another 10-fold for interindividual variability. The animal-to-human extrapolation can be subdivided into a factor of 4 for pharmacokinetics and a factor of 2.5 for interspecies variability in susceptibility [WHO 1994]. In this case, the interspecies pharmacokinetic factor is replaced by the use of the Gloede et al. [2011] pharmacokinetic model, leaving an interspecies UF of 2.5. The UF for interindividual variability can be subdivided into two factors of $\sqrt{10}$, or 3.2, one for interindividual variability in pharmacokinetics and the other for interindividual variability in susceptibility [WHO 1994]. Because the toxicity of diacetyl occurs at the point of contact with respiratory tract mucosa there is relatively little opportunity for interindividual variability in pharmacokinetics, and so the first subfactor is not applied. However, interindividual variability in susceptibility to toxicity cannot be ruled out; therefore, a factor of 3.2 is applied. In addition, a factor of 3 is applied for conversion from subchronic to chronic exposure. When the three factors (3.2-fold for interindividual variability, 2.5-fold for interspecies variability, and 3-fold for subchronic to chronic) are multiplied, the resulting total UF is 24.

6.2.2.7 Joint analysis of the data on mice from the diacetyl and 2,3-pentanedione bioassays

To avoid the loss of information inherent in dichotomizing ordinal data the severity scores of each type of lesion observed among nasal and lung tissues were modeled as having been sampled from conditional multinomial distributions given the unobserved random effects associated with each mouse described by the following family of complementary cumulative logistic models:

$$logit\left(Pr\left(Y_{bskcr(t)i} \geq j\right)\right) = log\left(\frac{Pr\left(Y_{bskcr(t)i} \geq j\right)}{1-Pr\left(Y_{bskcr(t)i} \geq j\right)}\right)$$
$$= \alpha_{sjr(t)} + u_{bskci} + \omega_s \cdot \tau_{bskci}$$
$$+ f_{bskcti}\beta_{sjr(t)}\{m(s, k, conc_{bskci}, t, \tau_{bskci}; \theta_{sr(t)}, \varphi_{skt}, \gamma_s)\} \cdot conc_{bskci},$$

where

$\alpha_{sjr(t)} + u_{skci} + \omega_s \cdot \tau_{bskci}$ describes effects in the absence of exposure,

$f_{bskcti}\beta_{sjr(t)}\{m(s, k, conc_{bskci}, t, \tau_{bskci}; \theta_{sr(t)}, \varphi_{skt}, \gamma_s)\} \cdot conc_{bskci}$ describes effects of exposure

and

b indexes the bioassay study

s indexes sex,

$k = 0 \leftrightarrow$ 2,3-pentanedione exposure and $k = 1 \leftrightarrow$ diacetyl exposure,

bkc identifies the exposure group and $conc_{bkc}$ is the corresponding exposure concentration,

$i = 1, \ldots, n_{bskc}$ indicates each of the mice within the exposure group identified by $bskc$ and $conc_{bskci}$ denotes the corresponding exposure concentration,

$r(t)$ identifies the response lesion, r nested within tissue, t, (lung or nasal),

$Y_{bskcr(t)i}$ is the response variable that is integer-valued based on the assigned severity score and it ranges over {0,1,2,3} for all response lesions[‡‡] except necrosis of the respiratory epithelium of the nose where the range was {0,1,2},

$Pr(Y_{bskcir(t)} \geq j)$ represents the expected proportion having response severity score greater than or equal to j for $j \in \{1, \ldots, \max(Y_{bskcr(t)i})\}$,

$\alpha_{sjt(r)}$ denotes the intercept parameters for lesion $r(t)$ which are subject to constraints

$\alpha_{s2t(r)} - \alpha_{s1t(r)} = \Delta\alpha_{s2} < 0$ and

$\alpha_{s3t(r)} - \alpha_{s2t(r)} = \Delta\alpha_{s3} < 0$ thus ensuring[§§] $\alpha_{s3t(r)} < \alpha_{s2t(r)} < \alpha_{s1t(r)}$,

$u_{bskci} \sim N(0, \sigma^2_{su})$ is a normally distributed random effect associated with the i^{th} mouse of $bskc$; likelihood ratio tests of null values of the variance parameters, σ^2_{us}, were performed[¶¶] and subject to being incorporated into the model.

$\omega_s \cdot \tau_{bskci}$ represents an adjustment to the intercepts allowing for effects associated with the longer durations quantified by τ_{bskci} of the diacetyl studies described by the unknown parameter, ω_s,

$\beta_{sjr(t)}$ are slope parameters for the effect exposure to 2,3-pentanedione, which are subject to constraints $\beta_{s2t(r)} - \beta_{s1t(r)} = \Delta\beta_{s2} \leq 0$ and $\beta_{s3t(r)} - \beta_{s2t(r)} = \Delta\beta_{s3} \leq 0$ thus ensuring[***] $\beta_{s3r(t)} \leq \beta_{s2r(t)} \leq \beta_{s1r(t)}$.

A test of $\Delta\beta_{s2} = \Delta\beta_{s3} = 0$ was performed[‡‡] and subjected to incorporation.

The slope parameters are subject to modification by the multiplicative function,

$m(s, k, conc_{bskci}, t, \tau_{bskci}; \emptyset_{sr(t)}, \varphi_{skt}, \gamma_s) = [1+\gamma_s \cdot \tau_{bskci}][1+I(k=1)\cdot(\emptyset_{sr(t)}-1) + \varphi_{skt} \cdot conc_{bskci}]$

where the factor,

$[1+\gamma_s \cdot \tau_{bskci}]$, describes an adjustment for the longer durations of the diacetyl studies parameterized by $\gamma_s > -1/max(\tau_{bskci})$; however, the assumption, $\gamma_s = \gamma$, was imposed because information was absent from female mice on this parameter, the diacetyl indicator, $I(k=1)=1$, when $k=1$ and $I(k=1)=0$ when k=0, $\emptyset_{sr(t)}$ are parameters describing the potency of diacetyl relative to 2,3-pentanedione

[‡‡]When no evidence of the lesion being modeled was detected a severity score of zero (0) was assigned.

[§§]Hence, the requirement that $r(Y_{kcit(r)} \geq 3) < Pr(Y_{kcit(r)} \geq 2) < Pr(Y_{kcit(r)} \geq 1)$ is satisfied for the controls.

[¶¶]Whenever the fitted values of the parameters were null, i.e., 0, the test statistic $-2\log(Likelihood\ ratio) = 0$ and the test was deemed to be nonsignificant.

[***]Hence, the requirement that $Pr(Y_{kcit(r)} \geq 3) < Pr(Y_{kcit(r)} \geq 2) < Pr(Y_{kcit(r)} \geq 1)$ is satisfied globally.

at low doses for {r(t)}; the hypothesis, $\emptyset_{sr(t)} = \emptyset_s$, was tested and subject to being incorporated into the model, and

φ_{skt} allows for an adjustment for a quadratic effect of concentration that may be attributed to directly proportional changes in respiratory ventilation with concentration where φ_{skt} is the constant of proportionality in units of controls' ventilation; thus φ_{skt} describes the change relative to controls. The hypothesis, $\varphi_{sk,lung} = \varphi_{sk,nose} = \varphi_{sk}$, was tested and subject to being incorporated into the model.

f_{bskcti} is one of a pair of lognormally distributed random effects (one effect per tissue indicated by t) of the i^{th} mouse of exposure group *bskc* acting multiplicatively on the effect of dose. Thus, an allowance for multiplicative variations from mouse to mouse by tissue-specific positive factors acting on the magnitudes of the slope parameters was incorporated. Each f_{bskcti} was modeled as having unit expectation and variance $(e^{\sigma_{st}^2}-1)$; thus, the variance of log $(f_{bskcti}) = \sigma_{st}^2$, $t = 1, 2$ for the *lung* and *nose*, respectively, together with an associated covariance parameter σ_{s12}. The hypothesis that lognormal random effects are independent was examined by testing $\sigma_{s12} = 0$ and was subject to being incorporated. Furthermore, the hypothesis that only one lognormal random effect for each mouse was necessary, i.e., $f_{bskc1i} \equiv f_{bskc2i}$ was tested and subject to being incorporated.

Model development proceeded by sequentially fitting a series of nested models of increasing complexity with all random effects omitted. This was advantageous for obtaining initial estimates of the fixed effects parameters for fitting a corresponding model that included random effects. Models were fitted by the method of maximum likelihood; the likelihoods of models containing unobserved random effects were obtained by integrating out these effects using adaptive Gaussian quadrature as described by Pinheiro and Bates [1995]. Likelihood ratio tests were performed to test hypotheses about model parameters and associated P values were based on the chi-square approximation to $-2\log$ *(LR)*. Evidence against incorporating the previously described restrictions on model parameters was deemed significant if the P value of the corresponding test was less than 0.05 for selecting the model on which to base the estimation of relative potency parameters and benchmark concentrations.

The model selected for estimation of relative potencies and BMCs contained three lognormal random effects parameters and 53 fixed-effects parameters; it had the following form:

$$logit\left(Pr\left(Y_{bskcr(t)i} \geq j\right)\right) = log\left(\frac{Pr\left(Y_{bskcr(t)i} \geq j\right)}{1-Pr\left(Y_{bskcr(t)i} \geq j\right)}\right)$$

$$= \alpha_{sjr(t)} + \omega_s \cdot \tau_{bskci} + f_{bskcti}\beta_{sr(t)}\{m(s,\ k,\ conc_{bskci},\ t,\ \tau_{bskci};\ \theta_{sr(t)},\ \varphi_{sk},\ \gamma)\} \cdot conc_{bskci}$$

where $m(s, k, conc_{bskci}, t, \tau_{bskci}; \theta_{sr(t)}, \varphi_{sk}, \gamma)$

$$= [1+\gamma \cdot \tau_{skci}]\ [1+I(k=1)\cdot(\theta_{sr(t)}-1)\ +\varphi_{sk} \cdot conc_{kci}]$$

i.e., this model was simplified by incorporating the following:

Null values of the variance parameters, σ_{ius}^2 [intercept random effects omitted],

$\Delta\beta_{s3} = \Delta\beta_{s2} = 0 \rightarrow \beta_{s3r(t)} = \beta_{s2r(t)} = \beta_{s1r(t)} = \beta_{sr(t)}$ [single 2,3-pentanedione slope parameter for each *sr(t)*],

Separate relative potency parameters, $\emptyset_{sr(t)}$ were retained since significant evidence against the hypothesis $\emptyset_{sr(t)} = \emptyset_s$ was obtained; hence, $\emptyset_{sr(t)}\beta_{sr(t)}$ describes the corresponding diacetyl slope for each *sr(t)*,

$\varphi_{sk,lung} = \varphi_{sk,nose} = \varphi_{sk}$ [quadratic effect independent of tissue],

$MLE(\sigma_{st}^2) = 0$ for lognormal random effects of nasal responses of female mice was replaced by nullifying this parameter,

The adequacy of a single lognormal random effect was rejected,

Independence of the lognormal random effects for lung and nasal tissues of male mice [implied by acceptance of $\sigma_{s12} = 0$] was assumed.

The model was coded and fitted using the NLMixed procedure of SAS™ 9.3. At least two lines of evidence provided support that the algorithm for fitting the model converged to a solution for the parameters that was a unique optimum as follows: (1) The Hessian matrix of the fit was positive definite[†††] and (2) exploration of the likelihood surface in a neighborhood of the solution via examination of likelihood profiles supported its optimality in all cases that were examined. Hence, this evidence supports the identifiability of the parameters of the model with these data suggesting that the model is not overparameterized.

The fit of the model was assessed by calculating grouped[‡‡‡] Pearson residuals conditional on the empirical Bayes estimates of the random effects $\left(\hat{\underline{f}}_{bskcti}^{(eB)}\right)$ for each tissue-response as follows:

$$r_{bskcr(t)j}^{cP} = \frac{\sum_{i\in bskcr(t)j} obs_{bskcr(t)ji} - \sum_{i\in bskcr(t)} \hat{E}\left(obs_{bskcr(t)ji}|\hat{\underline{f}}_{bskcti}^{(eB)}\right)}{\sqrt{\sum_{i\in bskcr(t)j} \hat{Var}\left(obs_{bskcr(t)ji}|\hat{\underline{f}}_{bskcti}^{(eB)}\right)}}$$

where the fitted expectations and variances of each mouse were based on the associated binomial distribution of a factoring of the conditional multinomial likelihood into its conditionally independent binomial components corresponding to the "outcomes" $(Y \geq 1 | \underline{f})$, $(Y \geq 2 | Y \geq 1, \underline{f})$, and $(Y \geq 3 | Y \geq 2, \underline{f})$.

Furthermore, a saturated fixed-effects model with random effects omitted was compared to the selected model by examination of twice the difference of log*(Likelihood)* values relative to the difference in the number of parameters. Finally, an ad hoc procedure was applied wherein binomial deviance residuals corresponding to factoring the multinomial likelihood of the corresponding 53 parameter model (with random effects omitted) into a product of conditional binomial terms were used to estimate a factor for adjusting the width of the confidence intervals analogous to an adjustment for overdispersion because the model-based confidence intervals may be too narrow if the model is incorrect. Two-sided 95% confidence limits with and without adjustment were calculated from application of a normal approximation to the natural logarithms of the relative potencies and the BMCs associated with a 10% benchmark response for additional risk.[§§§]

[†††]NLMixed minimizes –log(L) and it provides a warning if its criteria for a positive definite Hessian is not satisfied; no such warning was given.

[‡‡‡]The term "grouped" is to clarify that they are based on summing the observed and fitted expectations and variances over the mice within each treatment group defined by each unique combination of *b x k x s x c*.

[§§§]i.e., $Pr(Y_{skcr(t)} \geq j \mid conc=BMC_{jskr(t)}, f_{skcti} = 1) - Pr(Y_{skcr(t)} \geq j \mid conc = 0, f_{skcti} = 1) = 0.10$.

6.2.2.8 Benchmark concentration analysis using quantal models

To explore the impact of the categorical regression procedure described above on the BMC estimates for diacetyl, the data for the pathological endpoints listed in Table 6-1 (for rats) and Table 6-2 (for mice) were also dichotomized, and alternative benchmark concentration estimates were developed using quantal modeling and model averaging. Any response of minimal or greater severity was treated as a positive response, and the model averaging procedure was based on fitting the multistage, Weibull, and log-probit models, as described by Wheeler and Bailer [2007]. Only datasets with two or more partial response groups were modeled. The benchmark response rate was set at 10%, and the resulting BMC and BMCL estimates are shown in Table 6-9. Only models with an average-model *P* value of 0.05 or greater were considered to fit the data adequately.

6.3 Results

6.3.1 Diacetyl

BMC and BMCL estimates based on diacetyl toxicity in rats and mice were developed as described in sections 6.2.2.1 and 6.2.2.7, respectively. Not all of the pathological endpoints listed in Tables 6-1 and 6-2 could be adequately modeled. The rat endpoints that could be modeled adequately according to the criteria listed in section 6.2.2.1 (a score test for separate slopes and a likelihood ratio test for an unrestricted multinomial distribution) are shown in Table 6-6. Mouse endpoints that could be modeled adequately by the criteria described in section 6.2.2.7 are shown in Tables 6-7 and 6-8. The associated ventilation coefficient[""] of diacetyl among males was −0.378 ± 0.0582 and among females it was −0.530 ± 0.357.

The BMC and BMCL estimates were extrapolated to HECs as described in sections 6.2.2.2 – 6.2.2.4, and the HECs were converted to candidate REL values by the application of UFs as described in section 6.2.2.5. The BMC/BMCL values for rats, and their corresponding HEC and candidate REL values are shown in Table 6-6. The BMC/BMCL values for mice, and their corresponding HEC and candidate REL values are shown in Tables 6-7 and 6-8; the BMCL values in Table 6-7 have not been adjusted for overdispersion, while the BMCL values in Table 6-8 have been adjusted for overdispersion. Scatter plots of the 359 grouped Pearson residuals calculated from the data on mice indicated they were positively skewed at low concentrations and negatively skewed at high concentrations. Hence, they were not approximately normally distributed, which is to be expected given the discrete nature of the response data and the small numbers of mice in each treatment group ($5 \leq n \leq 10$). Although evidence of systematic departures of the residuals was not apparent, 13 of the residuals indicated deviations from the fit of the joint model of diacetyl and 2,3-pentanedione by more than three standard errors (not shown). Although less than one such residual deviation would be expected for normally distributed residuals the observation of 13 such deviations seems suggestive that extraneous variations may be present and motivated our having increased the widths of model-based confidence limits by the application of an overdispersion factor of 1.61 for adjusting the model-based standard errors.

Overall, the BMCs range from 16.8–68 ppm diacetyl, and the BMCLs range from 10–49.9 ppm diacetyl. After interspecies pharmacokinetic adjustments based on the Gloede et al. [2011] model, the human-equivalent BMCL values (BMCL_HECs) range from 1.4–95.8 ppm diacetyl, and the BMCL candidate REL

[""]Estimate of $\varphi_{s,diacetyl}$ ± Model-based standard error per 100 ppm.

values (after the application of uncertainty factors) range from 0.06–4.0 ppm diacetyl.

6.3.1.1 Sensitivity analysis

As a sensitivity analysis, alternative BMC and BMCL values were also derived for the NTP [2011] diacetyl study by dichotomizing the data, fitting quantal models, and model averaging, as described in section 6.2.2.8. The model average BMCs ranged from 14.6–78 ppm, with BMCLs of 2.4–57.9 ppm. The model average BMCs and BMCLs were extrapolated to humans as described above for the categorical-regression derived BMCs/BMCLs. The $BMCL_{HEC}$ values ranged from 0.9–54.3 ppm, and the $BMCL_{REL}$ values ranged from 0.04–2.26. As shown in Table 6-9, if the candidate RELs were derived from the quantal modeling rather than categorical regression, the lowest candidate REL value would be reduced from 0.06 ppm to 0.04 ppm.

Another assumption made in this risk assessment is that toxicity observed in mice can be scaled to rats using the EPA [1994] RfC methodology to estimate a mouse-to-rate respiratory dose ratio, or RGDR. It was assumed that this extrapolation is best performed on the basis of measured values of respiratory ventilation, as opposed to estimating respiratory ventilation on the basis of body weight. As detailed above in section 6.2.2.4, use of the measured respiratory ventilation rates leads to RGDRs of 2.4 for upper-respiratory toxicity and 3.2 for lower respiratory toxicity. The impact of the decision to use measured respiratory rates in the RGDR calculation was evaluated by a comparison to the RGDRs which would be obtained using the default RfC methodology, based on body weights, and described in EPA [1994]. Using the EPA [1994] default methodology, in which the respiratory ventilation rate is estimated from the animal biody weight, results in RGDRs of 1.15 for upper respiratory tract effects and 1.5 for lower respiratory tract effects. Therefore the mouse-to-rat scaling factor would be approximately halved, and as shown in Table 6-9 the lowest candidate REL value would be reduced to 0.03 ppm, based on chronic bronchial inflammation in the female mouse lung.

A key assumption made in this risk assessment is that the Gloede et al. [2011] PBPK model is the most appropriate method for extrapolating from rats to humans. A possible alternative would be to use the EPA [1994] RfC methodology to estimate animal-to-human scaling factors, based on the RGDR. Measured respiratory ventilation values are available for mice and rats, as used in section 6.2.2.4, and the human occupational respiration rate can be assumed to be 20 L/min. Using these values and the EPA [1994] procedures for category 1 gases, the estimated RGDRs for rat-to-human extrapolation are 0.18, 1.9, and 2.1 for the upper respiratory tract, the tracheobronchial region, and the pulmonary tract, respectively. Corresponding values for mouse-to-human extrapolation are 0.43, 5.9, and 6.9 for the upper respiratory tract, the tracheobronchial region, and the pulmonary tract, respectively. These RGDRs would replace the Gloede et al. PBPK model for extrapolating from rats to humans, and would result in candidate RELs ranging from 0.15–16.1 ppm for BMCs, and from 0.10–14.3 ppm for BMCLs. The lowest candidate REL derived using the RGDR method would be 0.10 ppm, as opposed to 0.06 ppm using the Gloede et al. [2011] model. The endpoints yielding the lowest alternative candidate REL values from the sensitivity analysis are shown in Table 6-9, along with the lowest of the candidate RELs from the main analysis, for comparison.

Table 6-6. Benchmark concentration* (BMC and BMCL) estimates, human-equivalent concentrations (HECs), and candidate recommended exposure limits based on toxicity in rats exposed to diacetyl

Sex	Tissue	Response	Separate slope *P* value†	Likelihood ratio *P* value‡	BMC (ppm)	BMCL (ppm)	Animal-to-human PK factor	BMC_{HEC} (ppm)	$BMCL_{HEC}$ (ppm)	UF	BMC_{REL} (ppm)	$BMCL_{REL}$ (ppm)
M	Lung	Infiltration cellular, histiocyte	0.49	0.45	43	30	15.70	2.7	1.9	24	0.11	0.08
M	Lung	Inflammation, eosinophil or acute	0.055	0.35	29	22	15.70	1.8	1.4	24	0.08	0.06
M	Nose	Olfactory epithelium, degeneration	0.59	0.95	20	13	0.66	30.3	19.7	24	1.26	0.82
M	Nose	Olfactory epithelium, metaplasia, respiratory	0.67	0.78	41	27	0.66	62.1	40.9	24	2.59	1.70
M	Nose	Olfactory epithelium, necrosis	0.23	0.62	27	19	0.66	40.9	28.8	24	1.70	1.20
M	Trachea	Epithelium, hyperplasia	0.28	0.81	68	47	8.70	7.8	5.4	24	0.33	0.23
F	Nose	Inflammation, suppurative	0.12	0.59	22	15	0.66	33.3	22.7	24	1.39	0.95
F	Nose	Lymphoid tissue, hyperplasia	0.83	0.20	23	18	0.66	34.8	27.3	24	1.45	1.14
F	Nose	Turbinate, atrophy	0.42	>0.99	36	24	0.66	54.5	36.4	24	2.27	1.52

*The benchmark concentration is based on a 10% benchmark response for a minimal or greater level of severity.

†Chi-square test *P* value for separate slopes for severity scores; $P > 0.05$ considered to indicate an adequate model fit by this criterion.

‡Chi-square test *P* value for a likelihood ratio test for an unrestricted multinomial distribution; $P > 0.05$ considered to indicate an adequate model fit by this criterion.

Table 6-7. Benchmark concentration* (BMC and BMCL) estimates, human-equivalent concentrations, and candidate recommended exposure limits based on toxicity in mice exposed to diacetyl; BMCLs not adjusted for overdispersion

Sex	Tissue	Response	BMC (ppm)	BMCL (ppm)	Animal-to-human PK factor	BMC_{HEC} (ppm)	$BMCL_{HEC}$ (ppm)	UF	BMC_{REL} (ppm)	$BMCL_{REL}$ (ppm)
M	Lung	Bronchus, inflammation, chronic	41.8	27.4	8.7	4.8	3.1	24	0.20	0.13
M	Lung	Bronchus, epithelium, regeneration	54.2	38.1	8.7	6.2	4.4	24	0.26	0.18
M	Nose	Inflammation, suppurative	30.5	24.7	0.28	109.0	88.2	24	4.54	3.68
M	Nose	Olfactory epithelium, atrophy	32.3	23.0	0.28	115.5	82.1	24	4.81	3.42
M	Nose	Respiratory epithelium, metaplasia, squamous	26.5	19.2	0.28	94.8	68.6	24	3.95	2.86
M	Nose	Respiratory epithelium, necrosis	36.0	26.8	0.28	128.5	95.9	24	5.35	4.00
M	Nose	Respiratory epithelium, regeneration	40.2	23.5	0.28	143.7	83.9	24	5.99	3.50
F	Lung	Bronchus, inflammation, chronic	19.4	15.3	8.7	2.2	1.8	24	0.09	0.08
F	Lung	Bronchus, epithelium, regeneration	56.1	49.9	8.7	6.5	5.7	24	0.27	0.24
F	Nose	Inflammation, suppurative	27.0	22.9	0.28	96.5	81.7	24	4.02	3.40
F	Nose	Olfactory epithelium, atrophy	22.0	17.2	0.28	78.5	61.4	24	3.27	2.56
F	Nose	Respiratory epithelium, metaplasia, squamous	21.8	17.8	0.28	77.7	63.7	24	3.24	2.65
F	Nose	Respiratory epithelium, necrosis	16.8	12.2	0.28	59.8	43.5	24	2.49	1.81
F	Nose	Respiratory epithelium, regeneration	18.7	13.4	0.28	66.6	47.8	24	2.78	1.99

*The benchmark concentration is based on a 10% benchmark response for a minimal or greater level of severity.

Table 6-8. Benchmark concentration* (BMC and BMCL) estimates, human-equivalent concentrations, and candidate recommended exposure limits based on toxicity in mice exposed to diacetyl; BMCLs adjusted for overdispersion

Sex	Tissue	Response	BMC (ppm)	BMCL (ppm)	Animal-to-human PK factor	BMC_{HEC} (ppm)	$BMCL_{HEC}$ (ppm)	UF	BMC_{REL} (ppm)	$BMCL_{REL}$ (ppm)
M	Lung	Bronchus, inflammation, chronic	41.8	21.2	8.7	4.8	2.4	24	0.20	0.10
M	Lung	Bronchus, epithelium, regeneration	54.2	30.8	8.7	6.2	3.5	24	0.26	0.15
M	Nose	Inflammation, suppurative	30.5	21.7	0.28	109.0	77.6	24	4.54	3.23
M	Nose	Olfactory epithelium, atrophy	32.3	18.7	0.28	115.5	66.7	24	4.81	2.78
M	Nose	Respiratory epithelium, metaplasia, squamous	26.5	15.8	0.28	94.8	56.3	24	3.95	2.35
M	Nose	Respiratory epithelium, necrosis	36.0	22.5	0.28	128.5	80.2	24	5.35	3.34
M	Nose	Respiratory epithelium, regeneration	40.2	16.9	0.28	143.7	60.5	24	5.99	2.52
F	Lung	Bronchus, inflammation, chronic	19.4	13.3	8.7	2.2	1.5	24	0.09	0.06
F	Lung	Bronchus, epithelium, regeneration	56.1	46.4	8.7	6.5	5.3	24	0.27	0.22
F	Nose	Inflammation, suppurative	27.0	20.7	0.28	96.5	73.9	24	4.02	3.08
F	Nose	Olfactory epithelium, atrophy	22.0	14.8	0.28	78.5	52.9	24	3.27	2.20
F	Nose	Respiratory epithelium, metaplasia, squamous	21.8	15.8	0.28	77.7	56.5	24	3.24	2.35
F	Nose	Respiratory epithelium, necrosis	16.8	10.0	0.28	59.8	35.9	24	2.49	1.50
F	Nose	Respiratory epithelium, regeneration	18.7	10.9	0.28	66.6	39.0	24	2.78	1.63

*The benchmark concentration is based on a 10% benchmark response for a minimal or greater level of severity.

Table 6-9. Alternate benchmark concentration (BMC and BMCL) estimates, human-equivalent concentrations, and candidate recommended exposure limits developed as a sensitivity analysis for key risk assessment assumptions

Species	Sex	Tissue	Response	BMC (ppm)	BMCL (ppm)	Animal-to-human PK factor	BMC_{HEC} (ppm)	$BMCL_{HEC}$ (ppm)	UF	BMC_{REL} (ppm)	$BMCL_{REL}$ (ppm)
Rat	Male	Lung	Eosinophilic inflammation*	29	22	15.7	1.8	1.4	24	0.08	0.06
Mouse	Female	Lung	Bronchus, inflammation, chronic†	19.4	13.3	8.7	2.2	1.5	24	0.09	0.06
Mouse	Female	Larynx	Chronic inflammation‡	16	2.4	2.7	5.9	0.9	24	0.25	0.04
Mouse	Male	Lung	Bronchus, inflammation, chronic§	19.4	13.3	18.7	1.0	0.7	24	0.04	0.03
Rat	Male	Nose	Olfactory epithelium, degeneration¶	20	13	0.18**	3.6	2.3	24	0.15	0.10

*From main analysis, Table 6-6, for comparison to alternate estimates.

†From main analysis, Table 6-8, for comparison to alternate estimates.

‡Alternative analysis based on quantal modeling.

§Alternative analysis using respiratory ventilation rates based on body weight, rather than measured values.

¶Alternative analysis using EPA [1994] RfC methodology rather than the Gloede et al. [2011] PBPK model.

**The animal-to-human PK factor shown here is the RGDR for the rat nose, which in EPA methodology is applied to the BMC/BMCL as a multiplicative factor. This is unlike the PBPK-derived PK factors above, which are applied as divisors for the BMC/BMCL values.

6.3.2 2,3-Pentanedione

The ventilation coefficient[****] of 2,3-pentanedione among male mice was −0.312 ± 0.0139 and among females it was −0.182 ± 0.0530. The relative potency estimates (diacetyl/2,3-pentanedione) are shown in Table 6-10, below, and range from 0.81–7.3, depending on sex and the specific endpoint evaluated. A relative potency of 1.00 indicates that the two compounds have equal toxic potency for the endpoints examined; a relative potency less than 1.00 indicates that 2,3-pentanedione is more toxic than diacetyl, while a relative potency greater than 1.00 indicates that 2,3-pentanedione is less toxic than diacetyl. Model-based 95% confidence limits range from 0.55–14, and the overdispersion-adjusted confidence limits range from 0.44–21. These estimates suggest that the potency of diacetyl was significantly greater than that of 2,3-pentanedione among female mice for these responses. However, one source of contribution to these estimates among females is that their fitted ventilation coefficient of diacetyl exposure was 2.9-fold of the coefficient fitted for 2,3-pentanedione exposure; thus, the observed responses were associated with substantially less diacetyl having been inhaled thereby increasing its fitted potency relative to 2,3-pentanedione. In contrast the corresponding value among males was 1.2. Furthermore, all seven estimates among females depended on the modeling assumption that the exposure duration parameter was identical to that of males and results of profiling the likelihood (not shown) illustrated that this dependence was unidimensional, i.e., the seven relative potency estimates for the females varied in unison with the duration parameter, whereas this was not the case for the seven parameter estimates of the males. Hence, the interpretation of the relative potency estimates among females warrants a substantially larger degree of caution. Although the majority of the relative potency estimates among male mice are greater than 1.0, suggesting that 2,3-pentanedione may be somewhat less toxic than diacetyl, two of the seven relative potency estimates (for olfactory epithelial atrophy and respiratory epithelial degeneration in the nasal tissues of male mice) are less than 1.0. In addition to these endpoints, the overdispersion-adjusted lower confidence limit estimates of relative potency for necrosis of the nasal respiratory epithelium, chronic bronchial inflammation and bronchial epithelial regeneration are also less than 1.0. Hence, these results suggest that equal or greater toxic potency for 2,3-pentanedione relative to diacetyl cannot be ruled out on the basis of currently available data.

[****]Estimate of $\varphi_{s,PD}$ ± Model-based standard error per 100 ppm

6.4 Discussion

6.4.1 Diacetyl

6.4.1.1 Modeling issues in BMC estimation for diacetyl

Categorical regression modeling for diacetyl BMC estimation was initially conducted as described in section 6.2.2.1 for rat and mouse data. However, it was noted that the mouse models showed systematic overprediction of the observed response at the highest exposure concentrations. Mice are well known to exhibit reduced respiration when exposed to respiratory irritants [Alarie and Stokinger 1973], including diacetyl [Larsen et al. 2009]. Reduced respiratory rate and reduced minute volume have been observed in male mice exposed to diacetyl [Morgan et al. 2008]. Speculatively, reduced respiration at high exposure concentrations may contribute to the attenuation of response noted in the high exposure groups, relative to a model where the effects of exposure are proportional to concentration. A strategy was therefore employed of modifying the model structure by including a quadratic dose term parameterized

Table 6-10. Relative potency estimates for diacetyl relative to 2,3-pentanedione, on the basis of data in male and female mice

Sex	Response	Relative potency (diacetyl/ 2,3-pentanedione)	Lower confidence limit*(model-based)	Upper confidence limit* (model-based)	Lower confidence limit (OD-adjusted)†	Upper confidence limit (OD-adjusted)**
F	Bronchus, inflammation, chronic	3.7	2.0	6.7	1.4	9.6
F	Bronchus, epithelium, regeneration	4.0	2.3	7.0	1.7	9.8
F	Nasal inflammation, suppurative	4.7	3.0	7.4	2.2	9.8
F	Olfactory epithelium, atrophy	2.0	1.4	2.9	1.1	3.7
F	Nasal respiratory epithelium, Metaplasia, squamous	7.3	3.8	14	2.5	21
F	Nasal respiratory epithelium, necrosis	3.5	2.2	5.3	1.7	6.9
F	Nasal respiratory epithelium, regeneration	2.9	1.6	5.3	1.1	7.7
M	Bronchus, inflammation, chronic	1.4	1.1	1.7	0.94	2.0
M	Bronchus, epithelium, regeneration	1.3	1.1	1.6	0.95	1.8
M	Nasal inflammation, suppurative	1.6	1.3	1.9	1.2	2.1
M	Olfactory epithelium, atrophy	0.89	0.70	1.1	0.60	1.3
M	Nasal respiratory epithelium, meta-plasia, squamous	1.5	1.2	1.8	1.0	2.1
M	Nasal respiratory epithelium, necrosis	1.4	1.0	1.9	0.84	2.2
M	Nasal respiratory epithelium, regeneration	0.81	0.55	1.2	0.44	1.5

*The upper and lower confidence limits form a 95% confidence limit for the relative potency estimate.

†Upper and lower confidence limits after adjusting for overdispersion, as described in section 6.2.2.7.

to represent directly proportional changes of ventilation with concentration in modeling the mouse data, which allowed sufficient model flexibility to accommodate the attenuation of response seen in the high-dose mouse data. The resulting coefficients of ventilation for nasal and lung tissues within each sex and exposure chemical were homogeneous and subsequently pooled. Furthermore, the coefficients of male mice for each chemical were similar and the diacetyl coefficient was consistent with the observations of minute volume by Morgan et al. [2008]. However, the coefficients of the two chemicals for the females were substantially dissimilar. The seven tissue responses of each mouse were jointly analyzed because they were governed by the same ventilation coefficient. To account for correlations among the responses, random effects were included in the model thereby utilizing all of the data for the estimation of parameters common to all responses. However, the increased complexity of the model in combination with the small sample sizes and discrete responses presented challenges for assessing its fit. Residuals were calculated conditional on estimates of the random effects but interpretations of these residuals based on their having an approximately normal distribution appeared to be problematic because a systematic relationship between their skewness and concentration was apparent. However, our interpretation of these residuals as providing evidence of deviations exceeding model-based predictions is prudent and motivated the increase of the widths of the confidence intervals. However, these modifications were not necessary in modeling the rat data, and were not included in the models developed for BMC estimation with the rat data.

In the current analysis, BMC estimates for diacetyl, based on categorical regression modeling, range from 16.8–68 ppm diacetyl, and the BMCL estimates range from 10–49.9 ppm diacetyl (Tables 6-6, 6-7, and 6-8). For comparison, alternative BMC estimates based on a quantal modeling range from 14.6–78 ppm, and quantal model BMCL estimates range from 2.4–57.9 ppm. Although the central BMC estimates were similar for the quantal and categorical modeling approaches, some of the quantal model BMCL estimates are several-fold lower than any obtained using categorical modeling. It is possible that this result may be due to the inclusion of additional information — response severity, as well as incidence — in the categorical regression modeling approach, leading to narrower confidence limits in comparison to the quantal modeling results. Additional sensitivity analyses explored the sensitivity of the toxicologically-based risk assessment for diacetyl to basing the mouse-to-rat extrapolation on allometrically-scaled respiration rates rather than measured values, and to basing the animal-to-human extrapolation on RfC methodology [EPA 1994] rather than the Gloede et al. [2011] PBPK model. As described in section 6.3.1.1, varying these assumptions would have relatively modest effects on the toxicologically-based REL estimate for diacetyl. As shown in Table 6-9, the lowest candidate REL values from the various sensitivity analyses are all within a factor of ±2 of the candidate REL values from the main analysis, suggesting that the value of the toxicologically-based candidate REL is not strongly dependent on these assumptions.

6.4.1.2 Comparison with other toxicologically-based risk assessments

The numerical values of BMD estimates for diacetyl are not all directly comparable, even when based on a common response rate of 10%, because of variations in the dose units used (ppm concentration versus regional penetration versus tissue concentration). The occupational exposure limits (OELs) developed by the various authors are directly comparable, but depend in part on assumptions regarding

uncertainty factors, which may vary between studies. In contrast, the HEC estimates derived in this analysis can be directly compared to the HEC estimates that have been developed in prior risk assessments.

Earlier toxicologically-based risk assessments of diacetyl [Allen 2009; Maier 2010] have been based on the 6-week and 12-week mouse study of Morgan et al. [2008], rather than the more extensive subchronic study conducted by the NTP [2011]. Because the NTP [2011] subchronic study included data from both mice and rats and included both more dose levels and more animals per dose group than the Morgan et al. [2008] study, the NTP [2011] diacetyl study was chosen as the basis for risk assessment in this document. However, comparison of the current risk assessment findings to the results of the earlier risk assessments is instructive. The HECs derived in prior diacetyl risk assessments are summarized in Table 6-11.

The BMC_{10} HEC estimates in the current study span a range of 1.8–143.7 ppm, compared to the range of 4.5–61 ppm reported in prior diacetyl risk assessments. The $BMCL_{10}$ HEC estimates in the current study span a range of 1.4–95.9 ppm, compared to the range of 1.3–10 ppm reported in prior diacetyl risk assessments. The wider range of HEC estimates in the current study, as compared to prior analyses, is partially due to the application of animal-to-human dosimetry estimates from the Gloede et al. [2011] PBPK/CFD model, which was published subsequent to the prior risk assessments and was, obviously, not available to prior risk assessors. In addition, the current study has the benefit of a more extensive toxicological data base for diacetyl because of publication of the NTP [2011] subchronic inhalation study, and therefore includes data from more pathological endpoints than the prior analyses did.

Maier et al. [2010] conducted a risk assessment for diacetyl for the purpose of deriving an OEL. This risk assessment was based on the mouse pilot study data of Morgan et al. [2008], using BMD methodology. The authors concluded that the most sensitive endpoint in the mouse

Table 6-11. HECs (ppm atmospheric concentration) corresponding to 10% BMDs and 10% BMDLs reported in prior diacetyl risk assessments

Study	Endpoint	Dose measure	BMD_{10} HEC (ppm)	$BMDL_{10}$ HEC (ppm)
Current study, categorical regression modeling	Various (Tables 6-6, 6-7, and 6-8)	Tissue concentration	1.8 – 143.7	1.4 – 95.9
Current study, quantal modeling	Various (Table 6-9)	Tissue concentration	3.1 – 95.7	0.9 – 54.3
Maier et al. [2010]	Peribronchial inflammation	Regional penetration	6.5	1.8
Allen [2009a]	Nasal inflammation	Regional penetration	61.0	10.4
Allen [2009a]	Nasal inflammation	Tissue concentration	4.5	3.0
Allen [2009a]	Peribronchial inflammation	Regional penetration	38.6	8.3
Allen [2009a]	Peribronchial inflammation	Tissue concentration	5.1	1.3
TERA [IDFA 2008]	Peribronchial inflammation	Regional penetration	9.0	2.0

was peribronchial lymphocytic inflammation. The authors estimated a $BMDL_{10}$ of 1.98 ppm diacetyl, which they converted to a HEC of 1.8 ppm, rounded to 2 ppm. The authors concluded that a total UF of 10 was appropriate, yielding in an OEL of 0.2 ppm.

A toxicologically-based quantitative risk assessment for diacetyl was conducted by Bruce C. Allen in the reports titled "A Quantitative Risk Assessment for Diacetyl Based on Respiratory Tract Lesions in Mice" [Allen 2009a] and "Report on Model Averaging Analysis and Results for Diacetyl Mouse Data Sets" [Allen 2009b] prepared under OSHA contract number DOLQ059622303 (2009) Task Order 50. These reports served as the basis for the toxicologically-based diacetyl risk assessment in the draft NIOSH criteria document for diacetyl in 2011 but have been supplanted in the current document by an analysis of more recent data. A summary of the risk assessment extracted from these reports is included here, for comparison to the current toxicologically-based quantitative risk assessment.

The [Allen 2009a] quantitative risk assessment was based on an analysis of adverse respiratory effects in mice exposed to diacetyl by inhalation 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. The Morgan et al. [2008] study was used to derive BMDs, BMDLs, and corresponding HECs, as discussed below. 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, 5 days/week, for either 6 or 12 weeks. The 6- and 12-week data were pooled for the final analysis, based on a likelihood ratio test that indicated that the 6- and 12-week results were not significantly different. A variety of dosimetric adjustments were considered in extrapolating the results from mice to humans. The most significant of these adjustments was the choice of dose metrics, either "regional penetration" (based on the percentage of diacetyl reaching a given portion of the respiratory tract), or "tissue concentration" (based on the Morris and Hubbs [2009] PBPK model). Because the choice of dose metrics has a significant impact on the HEC, and it is not clear which dose metric is preferable, HECs derived using both dose metrics are reported in Table 6-11. An assessment completed by Toxicology Excellence for Risk Assessment (TERA) [IDFA 2008] also utilized the dose-response data of Morgan et al. [2008], and estimated HECs based on BMDLs for 10% risk, comparable to those estimated in the current analysis. 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 contrast, the current assessment assumes that the dose-response relationship in a test species, rather than the lesion site, is the best criterion for choosing which endpoints to model for quantitative risk estimation. Thus, the current analysis assumes that site concordance is not a requirement because 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 differences in deposition of the chemical within the respiratory tracts of rodents and humans, as indicated by the PBPK model of Gloede et al. [2011]. The Gloede et al. [2011] model indicates that a much higher percentage of inhaled diacetyl reaches the bronchial and bronchiolar regions in humans than in rodents which provides a basis for the findings that diacetyl toxicity is observed primarily

in the upper respiratory tract of rodents and the lower respiratory tract of humans. TERA [IDFA 2008] estimated HECs using the EPA default methods [EPA 1994] modified by the PBPK/CFD model predictions of Morris and Hubbs [2009]. However, rather than using the relationships between the default and CFD-model-predicted scrubbing factors to define a mouse-specific estimate of airway scrubbing of diacetyl, 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). The TERA [IDFA 2008] risk assessment did not consider light exercise conditions, as may occur in the workplace, as these were not incorporated into the PBPK/CFD modeling of Morris and Hubbs [2009]. Moreover, for the effective dose (regional penetration) measure calculated by TERA, the default mouse ventilation rates were used. As discussed above in regard to the Allen [2009a] risk assessment, the experimentally measured ventilation rates for the Morgan et al. [2008] study were substantially greater than the EPA default values (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, V_E/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]. The TERA assessment suggested that a composite uncertainty factor of 10 should be used to adjust those HECs downward to an 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).

6.4.2 2,3-Pentanedione

Toxic potency estimation for 2,3-pentanedione is constrained by both the limited numbers of animals that have been tested and the differing exposure durations used in the diacetyl and 2,3-pentanedione studies. The currently available histopathological data for repeated exposures to 2,3-pentanedione are limited to a single study involving exposures of 2 weeks + 2 days (totaling 12 exposures per animal), in both rats and mice. The rat data and female mouse data for diacetyl are limited to a single 13-week study [National Toxicology Program 2011], so that no data on the relationship of toxicity to duration of exposure are available for the rat or the female mouse. For male mice, limited data are available from the 6- and 12-week exposures reported by Morgan et al. [2008]. Although no mouse studies are available that closely approximate the 2 week + 2 day exposure protocol used in the 2,3-pentanedione study, the 6-, 12-, and 13-week diacetyl data on male mice were used to estimate an adjustment to predict what the toxicity of diacetyl would have been in a study of the same duration as the 2,3-pentanedione study. Although a small increase of toxicity with exposure duration was fitted it was retained in the model even though it was not significant in order to account for it as a source of variation in obtaining the standard errors of the seven relative potency estimates[††††] of each sex. The resulting relative potency estimates suggest that 2,3-pentanedione may have equal or greater toxic potency than diacetyl for five of the seven responses of male mice from

[††††]For those readers acquainted with the concept of Stein estimation for adjusting a set of three or more estimates an application of a criterion of Bock [1975] to the covariance matrix of each set did not support making them.

Table 6-10. Although the responses of Table 6-10 superficially suggest that 2,3-pentanedione is or seems to be less toxic than diacetyl to female mice, these estimates are sensitively dependent on the assumption that the parameter for exposure duration is identical to that of males. Furthermore, there is a complete lack of information from these studies for assessing this assumption and profiling the likelihood indicated that the relative potency estimates of the female mice were substantially sensitive to this parameter whereas this did not hold for the estimates of the males. Hence, it would be prudent to refrain from concluding that 2,3-pentanedione is less toxic than diacetyl to female mice on the basis of the estimates of Table 6-10.

Recent data support the conclusion that 2,3-pentanedione should be used cautiously in the workplace and exposures to 2,3-pentanedione should be minimized. Rats (but not mice) develop intramural and intraluminal airway fibrosis following exposure to either diacetyl or 2,3-pentanedione [Morgan et al. 2016]. This lesion shares many features with obliterative bronchiolitis of humans, the condition that originally brought medical attention to employees exposed to diacetyl. A 2-week inhalation exposure of 150 or 200 ppm to either diacetyl or 2,3-pentanedione could produce bronchial fibrosis in rats [Morgan et al. 2016]. This finding suggests that 2,3-pentanedione causes airway fibrosis comparable to diacetyl at equal exposure concentrations. Because no chronic or subchronic studies of 2,3-pentanedione are currently available and the number of rats in the 2-week exposure is low, it is not possible to quantitatively assess the toxicity of 2,3-pentanedione relative to diacetyl for producing airway fibrosis. However, these data do suggest that it would be prudent to treat 2,3-pentanedione as at least equally toxic as diacetyl until additional toxicological data become available on the toxic potency of 2,3-pentanedione.

6.5 Conclusions

Pathological lesions produced by inhalation exposure to diacetyl and 2,3-pentanedione have been assessed using categorical regression techniques and benchmark dose estimation. For diacetyl a CFD/PBPK model is available for both rats and humans that allows rodent BMC and BMCL estimates to be extrapolated directly to human exposures. The results of this exercise indicate that the most sensitive endpoint in terms of estimated human toxicity is that associated with eosinophilic inflammation in the male rat lung. The HEC associated with this endpoint is 1.8 ppm, with a 95% lower-bound estimate of 1.4 ppm (Table 6-6). Application of a 24-fold uncertainty factor to the lower-bound HEC leads to a candidate REL of 0.06 ppm, or 60 ppb diacetyl. The estimated human toxicity based on chronic bronchial inflammation in the female mouse lung is very similar to the rat-based estimate (Table 6-8), and also leads to a candidate REL of 0.06 ppm or 60 ppb. If human data on the toxicity of diacetyl were not available, these estimates could serve as the bases for REL development for diacetyl. Because human data do exist and are sufficient for derivation of an REL, the toxicologically-based candidate RELs should be viewed as complementary to the epidemiologically-based REL. Because the toxicologically-based REL is within an order of magnitude of the epidemiologically-based REL it supports the epidemiologically-based REL.

References

Alarie Y, Stokinger HE [1973]. Sensory irritation by airborne chemicals. CRC critical reviews in toxicology *2*(3):299–363.

Allen BC [2009a]. A quantitative risk assessment for diacetyl based on respiratory tract lesions in mice. Chapel Hill, NC: Prepared for the Occupational Safety and Health Administration, Prime Contract Number DOLQ05622303.

Allen BC [2009b]. Report on model averaging analysis and results for diacetyl mouse data sets. Chapel Hill, NC: Prepared for the Occupational Safety and Health Administration, Prime Contract Number DOLQ05622303.

Crump KS [1984]. A new method for determining allowable daily intakes. Fundam Appl Toxicol *4*(5):854–871.

EPA (Environmental Protection Agency) [1994]. Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. http://www.epa.gov/raf/publications/methods-derivation-inhalation-ref.htm. Date accessed: March 2011.

EPA [2012]. Benchmark Dose Technical Guidance. Risk Assessment Forum. http://www.epa.gov/raf/publications/pdfs/benchmark_dose_guidance.pdf. Date accessed: August 2014.

Gloede E, Cichocki JA, Baldino JB, Morris JB [2011]. A validated hybrid computational fluid dynamics-physiologically based pharmacokinetic model for respiratory tract vapor absorption in the human and rat and its application to inhalation dosimetry of diacetyl. Toxicol Sci *123*(1):231–246.

Grote A, Castranova V, Kullman G, Fedan JS, Dowdy J, Jones WG [2002]. Necrosis of nasal and airway epithelium in rats inhaling vapors of artificial butter flavoring. Toxicol Appl Pharmacol *185*(2):128–135.

Guth DJ, Carroll RJ, Simpson DG, Zhou H [1997]. Categorical regression analysis of acute exposure to tetrachloroethylene. Risk Anal *17*(3):321–332.

Haber L, Strickland JA, Guth DJ [2001]. Categorical regression analysis of toxicity data. Comments on toxicology *7*(5-6):437–452.

Hubbs AF, Battelli LA, Goldsmith WT, Porter DW, Frazer D, Friend S, Schwegler-Berry D, Mercer RR, Reynolds JS, Hubbs AF, Goldsmith WT, Kashon ML, Frazer D, Mercer RR, Battelli LA, Kullman GJ, Schwegler-Berry D, Friend S, Castranova V [2008]. Respiratory toxicologic pathology of inhaled diacetyl in Sprague-Dawley rats. Toxicol Pathol *36*(2):330–344.

ICRP [1994]. Human respiratory tract model for radiological protection. ICRP Publication 66 Ann ICRP *24*(1–3).

IDFA (International Dairy Foods Association) [2008]. International Dairy Foods Association. OSHA Docket Number OSHA-2008-0046.

Larsen ST, Alarie Y, Hammer M, Nielsen GD [2009]. Acute airway effects of diacetyl in mice. Inhal Toxicol *21*(13):1123–1128.

Maier A, Kohrman-Vincent M, Parker A, Haber LT [2010]. Evaluation of concentration–response options for diacetyl in support of occupational risk assessment. Regulatory Toxicology and Pharmacology *58*(2):285–296.

McCullagh P [1980]. Regression models for ordinal data. be J R Stat Soc Series B Stat Methodol:109–142.

Morgan DL, Flake GP, Kirby PJ, Palmer SM [2008]. Respiratory toxicity of diacetyl in C57BL/6 mice. Toxicol Sci *103*(1):169–180.

Morgan DL, Jokinen MP, Price HC, Gwinn WM, Palmer SM, Flake GP [2012]. Bronchial and bronchiolar fibrosis in rats exposed to 2,3-pentanedione vapors: implications for bronchiolitis obliterans in humans. Toxicol Pathol *40*(3):448–465.

Morgan DL, Jokinen MP, Johnson CL, Price HC, Gwinn WM, Bousquet RW, Flake GP [2016]. Chemical reactivity and respiratory toxicity of the alpha-diketone flavoring agents: 2,3-butanedione, 2,3-pentanedione, and 2,3-hexanedione. Toxicol Pathol *44*(5):763–783.

Morris JB, Hubbs AF [2009]. Inhalation dosimetry of diacetyl and butyric acid, two components of butter flavoring vapors. Toxicol Sci *108*(1):173–183.

National Toxicology Program [2011]. Short-term bioassay data for 2,3-butanedione (diacetyl). http://tools.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=shorttermbioassaydata.datasearch&chemical_name=2%2C3%2DButanedione%20%28Diacetyl%29&cas_no=431-03-8&study_no=C20725&study_length=90%20Days&protocol_no=2072502. Date accessed: 7/25/2011.

Pinheiro JC, Bates DM [1995]. Approximations to the log-likelihood function in the nonlinear mixed-effects model. J Comp Graph Stat *4*(1):12–35.

Wheeler MW, Bailer AJ [2007]. Properties of model-averaged BMDLs: a study of model averaging in dichotomous response risk estimation. Risk Anal *27*(3):659–670.

WHO [1994]. Environmental Health Criteria 170. Assessing human health risks of chemicals: Derivation of guidance values for health-based exposure limits. International Programme on Chemical Safety, Geneva, Switzerland.

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 employee 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. The primary objective of the recommendations for diacetyl is to reduce loss of lung function associated with diacetyl exposure because diacetyl (and potentially related diones) has been shown to cause potentially fatal obliterative bronchiolitis in employees. The NIOSH REL for 2,3-pentanedione would be identical to that for diacetyl but is slightly higher based upon the limitations of the analytical method.

7.1 Health Effect Studies of Employees Exposed to Diacetyl

As detailed in Chapter 3, medical evaluations showed that employees exposed to diacetyl developed progressive shortness of breath while working at several microwave popcorn plants and flavoring plants, findings consistent with the severe irreversible lung disease obliterative bronchiolitis. Obliterative bronchiolitis, sometimes characterized by spirometric abnormality, has been described in employees in the microwave popcorn and flavor-manufacturing industries [CDC 2002, 2007; Kanwal et al. 2006]. Some affected employees have experienced extremely rapid declines in lung function, with severe airways obstruction in some cases occurring within several months of the start of exposure to flavoring compounds [Akpinar-Elci et al. 2004; NIOSH 1986]. Employees as young as 22 years old have been affected. Some affected employees 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 established a link between exposure to diacetyl and risk for severe occupational lung disease. These findings meet the standard criteria used to determine causation: that an exposure is the likely cause of specific health effects [Gordis 1996; Hill 1965]. Investigations of severe lung disease consistent with obliterative bronchiolitis among diacetyl-exposed employees have provided clear 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 ≥290 ppm and caused epithelial necrosis and inflammation in the trachea and larynx at concentrations of ≥220 ppm [Hubbs et al. 2008]. In a pattern similar to that 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 at higher regions 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. The animal-based risk assessment presented in Chapter 6 further corroborates 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 employees 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 (employee) 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 employee 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 incidence of 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 5-35). Excess lifetime risks in the range of 1:1,000 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 respiratory impairment (decreased lung function) and the severe irreversible lung disease obliterative bronchiolitis associated with occupational exposure to these compounds. 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 employees. 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, however, additional considerations included sampling and analytical feasibility and the achievability of engineering controls.

A variety of risk estimates were evaluated and presented in Chapter 5. NIOSH has historically targeted excess risks predicted to be in the range of approximately 1 per 1,000 in establishing RELs (see Chapter 5, Tables 5-34, 5-35 for risk estimates). In occupational exposure to diacetyl, the ultimate health effect of concern is obliterative bronchiolitis, a debilitating, sometimes fatal, and irreversible effect. The goal is to prevent the respiratory impairment that precedes the appearance of obliterative bronchiolitis. There are validated analytical methods that can be used to effectively measure employee exposures at the selected level. Additionally, information from site visits indicates that the REL is achievable with engineering controls where diacetyl is used or handled [Eastern Research Group 2009; Kanwal et al. 2011].

7.5 Recommended Exposure Limits

7.5.1 Recommended Exposure Limit for Diacetyl

On this basis, NIOSH recommends a REL of 5 ppb for diacetyl (as a TWA for up to 8 hours/day during a 40-hour workweek). NIOSH has determined that employees 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 1/1,000 excess risk of lung function falling below the lower limit of normal due to diacetyl exposure.

To ensure that employee 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 corrective 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 employee exposures (described in Chapter 10) will help protect employees.

NIOSH is also recommending a STEL for diacetyl of 25 ppb for a 15-minute time period. The establishment of a short-term exposure limit 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 employee exposures to diacetyl. The selection of a STEL that is five times the REL is based upon past precautionary practice [Federal Register 1997]. In the absence of a STEL in workplaces complying with the NIOSH REL for diacetyl of 5 ppb TWA, employees could theoretically be exposed to 2,400 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 and should prevent acute irritation from brief high exposures.

7.5.2 Recommended Exposure Limit for 2,3-Pentanedione

2,3-Pentanedione, which has been used as a substitute for diacetyl, is also of concern because of structural similarities with diacetyl and because animal studies show similar toxicity for the respiratory tract [Hubbs et al. 2012; Morgan et al. 2012; Morgan et al. 2016]. Morphologic data suggest that 2,3-pentanedione can cause airway epithelial damage similar to the damage caused by diacetyl [Hubbs et al. 2012; Morgan et al. 2012; Morgan et al. 2016]. Rats repeatedly inhaling 2,3-pentanedione at concentrations ≥ 150 ppm for up to 2 weeks develop fibrosis of intrapulmonary airways, a morphologic change similar to obliterative bronchiolitis in humans [Morgan et al. 2016]. Recently, more than 3500 genes were found to be upregulated in RNA isolated from the fibrotic bronchi of 2,3-pentanedione exposed rats [Morgan et al. 2015]. Some of the up-regulated genes were ones previously implicated in fibrosis, including transforming growth factor-β2, interleukin-1α, interleukin-18, interleukin-33, and fibronectin. In addition, at high exposure concentrations, messenger RNA changes were noted in the brain of rats after acute 2,3-pentanedione inhalation [Hubbs et al. 2012].

The toxic potency of the two materials appears to be comparable in mice exposed by inhalation (see Chapter 6, section 2 for a full discussion). Given the structural similarity between diacetyl and 2,3-pentanedione and the evidence published, 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 concentrations 9.3 ppb and above. Therefore the NIOSH REL for 2,3-pentanedione, while informed by the toxicological potential, is based upon the limitations 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 similar 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 employees.

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 engineering controls, exposure monitoring, routine medical surveillance, and employee 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 lifetime 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 NIOSH believes there is insufficient historical information and statistical power to implement. Furthermore, there is no precedent for developing standards that are specific to smoking status. NIOSH also recommends an AL of 2.6 ppb to help protect employees 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 employee 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 employees. Many of the processes 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 compounds in liquid and powder form. A 3-year study of a microwave popcorn production facility showed that the use of exposure controls can dramatically reduce diacetyl concentrations in mixing rooms and for all production employees [Kanwal et al. 2011]. As a result of the implementation of exposure controls, average combined personal and area diacetyl air concentrations declined an order of magnitude in the mixing room (from 57.2 ppm to 2.88 ppm) while concentrations in the quality control laboratory (from 0.82 ppm to < LOD) and packaging area (from 2.76 ppm to < LOD for machine operators) declined to below detectable limits. These interventions included providing general room exhaust ventilation to the mixing room and local exhaust ventilation for the heated flavoring and mixing tanks. Closed transfer processes were implemented through the installation of a pump to transfer heated butter flavorings from the holding tanks to oil/flavor mixing tanks. The building of an enclosure for all oil/flavor holding tanks and installing local exhaust ventilation on all tanks further reduced exposures to employees in the packaging area of this plant. In the final survey conducted following the implementation of all engineering and process controls, personal diacetyl exposures for all employees/job categories in the plant were below detectable limits

with the exception of mixers which ranged from below the LOD to 12.6 ppm.

The design concepts required for working with hazardous materials include specification of general ventilation, local exhaust ventilation, 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 compounds; coffee roasting; commercial fry-cooking) may also result in employee exposure. Special attention should be given to manual handling of flavoring compounds, particularly in heated processes, and when spraying flavoring compounds. Research on food industry practices has led to the development of engineering controls that may help reduce employee 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. Table 8-2 in Chapter 8 provides a summary of NIOSH evaluated engineering control efficiencies for the mixing of food flavorings.

Although many job categories can be effectively controlled to levels below the REL, tasks associated with transfer of diacetyl may continue to pose risk to the employees even following the implementation of controls. For example, mixers may continue to be exposed at levels above the REL when handling butter flavorings and from tank emissions. However, these exposures can be reduced through the implementation of closed transfer systems and local exhaust ventilation approaches discussed in Chapter 8. NIOSH acknowledges that the frequent use of personal protective equipment, including respirators, may be required for some employees 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) airborne concentrations of diacetyl or 2,3-pentanedione (e.g., pouring, mixing, packaging) above the REL 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 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 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 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 structures similar 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 concentrations as low as possible, taking into account potential additive effects of flavoring compounds.

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 compounds used in this industry. The control of exposures is discussed in detail in Chapter 8, but several LEV systems described have been shown to be particularly effective in controlling diacetyl and would be expected to work well for similar compounds. Ventilated backdraft 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 employee exposure during pouring and mixing of ingredients in a commercial mixer has been evaluated in a flavoring production plant [NIOSH 2008b]. 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 an employee during the mixing of a food flavoring [NIOSH 2008b]. The use of the ventilated tank lid resulted in a reduction of approximately 76% exposure. 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 (HSE) to contain vapors during the mixing of liquids with other liquids or solids [Health and Safety Executive 2003].

7.9 Summary

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

- Airborne exposures to diacetyl and 2,3-pentanedione have been characterized as potentially hazardous based on 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 obliterative bronchiolitis. Studies show a progressive shortness of breath for employees at several microwave popcorn plants and flavoring plants as well as employees who have experienced rapid declines in lung function.
- Rats repeatedly inhaling 2,3-pentanedione at concentrations ≥ 150 ppm for up to 2 weeks develop fibrosis of intrapulmonary airways, a morphologic change similar to obliterative bronchiolitis in humans. Inhalation studies on mice produced similar results.
- Risk assessment 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 to achieve a 1/1,000 excess lifetime risk. 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 employee 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 evidence that 2,3-pentanedione can cause airway epithelial damage 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 does not imply that 2,3-pentanedione is of lower toxicity than diacetyl. Further, NIOSH recommends a STEL of 31 ppb 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 employee exposures at these levels.

References

Akpinar-Elci M, Travis WD, Lynch DA, Kreiss K [2004]. Bronchiolitis obliterans syndrome in popcorn production plant workers. Eur Respir J *24*(2):298–302.

CDC (Centers for Disease Control and Prevention) [2002]. Fixed obstructive lung disease in workers at a microwave popcorn factory—Missouri, 2000–2002. MMWR *51*(16):345–347.

CDC (Centers for Disease Control and Prevention) [2007]. Fixed obstructive lung disease among workers in the flavor-manufacturing industry—California, 2004–2007. MMWR *56*(16):389–393.

Eastern Research Group, Inc. [2009]. Site visits related to diacetyl and flavorings that contain diacetyl: food production facility G—buttered popcorn production (pre-popped). OSHA Docket No. 2008–0046–0081. By Eastern Research Group, Inc. Lexington, MA.

Epperly BR, Dekker EE [1989]. Inactivation of Escherichia coli L-threonine dehydrogenase by 2,3-butanedione. Evidence for a catalytically essential arginine residue. J Biol Chem *264*(31):18296–18301.

Federal Register [1997]. *62*(7):1577.

Gloede E, Cichocki JA, Baldino JB, Morris JB [2011]. A validated hybrid computational fluid dynamics-physiologically based pharmacokinetic model for respiratory tract vapor absorption in the human and rat and its application to inhalation dosimetry of diacetyl. Toxicol Sci *123*(1):231–246.

Gordis L [1996]. Epidemiology. Philadelphia, PA: W. B. Saunders Company, pp. 176–177.

Health and Safety Executive [2003]. Mixing liquids with other liquids or solids. In: COSHH essentials: easy steps to control chemicals. London: Health and Safety Executive.

Hill AB [1965]. The environment and disease: association or causation? Proc R Soc Med *58*:295–300.

Hubbs AF, Battelli LA, Goldsmith WT, Porter DW, Frazer D, Friend S, Schwegler-Berry D, Mercer RR, Reynolds JS, Grote A, Castranova V, Kullman G, Fedan JS, Dowdy J, Jones WG [2002]. Necrosis of nasal and airway epithelium in rats inhaling vapors of artificial butter flavoring. Toxicol Appl Pharmacol *185*(2):128–135.

Hubbs AF, Goldsmith WT, Kashon ML, Frazer D, Mercer RR, Battelli LA, Kullman GJ, Schwegler-Berry D, Friend S, Castranova V [2008]. Respiratory toxicologic pathology of inhaled diacetyl in Sprague-Dawley rats. Toxicol Pathol *36*(2):330–344.

Hubbs AF, Cumpston AM, Goldsmith WT, Battelli LA, Kashon ML, Jackson MC, Frazer DG, Fedan JS, Goravanahally MP, Castranova V, Kreiss K, Willard PA, Friend S, Schwegler-Berry D, Fluharty KL, Sriram K [2012]. Respiratory and olfactory cytotoxicity of inhaled 2,3-pentanedione in Sprague-Dawley rats. Am J Pathol *181*(3):829–844.

Kanwal R, Kullman G, Fedan K, Kreiss K [2011]. Occupational lung disease risk and exposures to butter flavoring chemicals after implementation of controls at a microwave popcorn plant. Public Health Rep *126*:480–494.

Kanwal R, Kullman G, Piacitelli C, Boylstein R, Sahakian N, Martin S, Fedan K, Kreiss K [2006]. Evaluation of flavorings-related lung disease risk at six microwave popcorn plants. J Occup Environ Med *48*(2):149–157.

Miller AG, Gerrard JA [2005]. Assessment of protein function following cross-linking by alpha-dicarbonyls. Ann NY Acad Sci *1043*:195–200.

Morgan DL, Flake GP, Kirby PJ, Palmer SM [2008]. Respiratory toxicity of diacetyl in C57BL/6 mice. Toxicol Sci *103*(1):169–180.

Morgan DL, Jokinen MP, Price HC, Gwinn WM, Palmer SM, Flake GP [2012]. Bronchial and bronchiolar fibrosis in rats exposed to 2,3-pentanedione vapors: implications for bronchiolitis obliterans in humans. Toxicol Pathol *40*(3):448–465.

Morgan DL, Jokinen MP, Johnson CL, Price HC, Gwinn WM, Bousquet RW, Flake GP [2016]. Chemical reactivity and respiratory toxicity of the alpha-diketone flavoring agents: 2,3-butanedione, 2,3-pentanedione, and 2,3-hexanedione. Toxicol Pathol *44*(5):763–783

Morris JB, Hubbs AF [2009]. Inhalation dosimetry of diacetyl and butyric acid, two components of butter flavoring vapors. Toxicol Sci *108*(1):173–183.

Naumann BD, Sargent EV, Starkman BS, Fraser WJ, Becker GT, Kirk GD [1996]. Performance-based exposure control limits for pharmaceutical active ingredients. AIHA J *57*(1):33–42.

NIOSH [1986]. Hazard evaluation and technical assistance report: International Bakers Services, Inc., South Bend, IN. By McConnell R, Hartle R. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 1985–0171–1710.

NIOSH [2008a]. Evaluating occupational exposures and work practices at Agilex Flavors, Inc. formerly Key Essentials, Inc., Rancho Santa Margarita, CA. A technical assistance report to the California/Occupational Safety and Health Administration. By McKernan L, Dunn K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2006–0361–2.

NIOSH [2008b]. In-depth survey report: evaluation of engineering controls for the production of liquid and powder flavoring chemicals. Kerry Ingredients and Flavours, Inc., formerly Mastertaste, Inc., Commerce, CA. By Dunn K, Echt A, Couch J, Myers D. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH EPHB Report No. 322–12a.

Palmer SM, Flake GP, Kelly FL, Zhang HL, Nugent JL, Kirby PJ, Foley JF, Gwinn WM, Morgan DL [2011]. Severe airway epithelial injury, aberrant repair and bronchiolitis obliterans develops after diacetyl instillation in rats. PLoS One *6*(3):e17644.

Roberts DW, York M, Basketter DA [1999]. Structure-activity relationships in the murine local lymph node assay for skin sensitization: alpha,beta-diketones. Contact Dermatitis *41*(1):14–17.

Saraiva MA, Borges CM, Florencio MH [2006]. Non-enzymatic model glycation reactions: a comprehensive study of the reactivity of a modified arginine with aldehydic and diketonic dicarbonyl compounds by electrospray mass spectrometry. J Mass Spectrom *41*(6):755–770.

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

8.1 Introduction

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

- Elimination/Substitution
- Engineering controls
- Administrative controls (including work practices)
- Personal protective equipment

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 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 other 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 may also need to be controlled. Therefore, elimination and substitution may 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 compounds 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 minimal user effort or training.

Work practices are procedures followed by employers and employees 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 compounds 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. NIOSH has recently published additional engineering and work practice control guidance for employees who are exposed to diacetyl [NIOSH 2015].

The use of respirators, a form of PPE, 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 also 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.

8.2.1 General Considerations

A properly designed supply air ventilation system can provide plant ventilation, building pressurization, and exhaust air replacement. When 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 2013].

Controls need to be fitted to individual processes by each plant and cannot be a "one-size-fits-all" approach. Controls need to be evaluated after installation. Evaluations should be completed to quantify exposures after controls have been implemented to ensure that target goals have been achieved. 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 ACGIH Operation and Maintenance Manual recommends the installation of a fixed hood static pressure gauge [ACGIH 2007].

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 preventive 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 compounds 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. Check status with airflow indication equipment such as a smoke tube.

- Install hood static pressure gauges (manometers) near hoods to provide a way to verify proper hood performance. Check pressure frequently to ensure that the system is operating properly compared to baseline. Check hood face velocities and capture velocities frequently to ensure that system is performing as designed.
- Ensure that employees are properly trained on the use of the controls if using proximity switches for fan activation. Consider installing a control "on/off" light to indicate the status of the exhaust fan.
- Place hoods away from doors, windows, air supply registers, and aisles when possible 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.
- Inspect hoods and enclosures for signs of damage or leaks (rust/corrosion, open access doors, etc.) and obstructions (paper, gloves, rags, etc.). Where possible, use screens to prevent foreign objects from being pulled into the system through openings (slots, hood faces, etc.).

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 employee exposure to diacetyl, 2,3-pentanedione and other flavoring compounds. 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 2008b]. Employees in each of these job categories may potentially be exposed to flavoring compounds, 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 compounds. 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 PPE posted for that specific area. Additional considerations

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(s)
Production operator	Benchtop weighing and handling	8.2.2.1	8-1, 8-2, 8-3, 8-4
	Charging/filling tanks and mixers	8.2.2.2	8-5, 8-6, 8-7, 8-8
	Bag dumping/emptying	8.2.2.3	8-9
	Drum filling and emptying	8.2.2.4	8-10, 8-11
Packaging personnel	Bag filling	8.2.2.5	8-12, 8-13
	Drum filling and emptying	8.2.2.4	
Quality assurance/ quality control personnel	Benchtop weighing and handling	8.2.2.1.1	8-4

may be necessary for the maintenance job category, specifically for intermittent tasks such as filter change out.

Many different industries have implemented engineering controls to reduce exposure and risk of disease among their employees. 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 compounds 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 compounds) may also result in employee exposure. Special attention should be given to manual handling of flavoring compounds, particularly in heated processes, and when spraying flavoring compounds.

Research into various food industries has led to the development of potential engineering controls to help reduce employee 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 employee 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 dry and wet food ingredients can lead to employee exposure primarily through the scooping, pouring, and dumping of these materials. Studies in bakeries have shown that the employees 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 2008a]. 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 employee exposure to chemicals during mixing and weighing has been evaluated in flavoring production. In flavoring production facilities, compounders measure and pour flavoring compounds 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) has been evaluated by NIOSH in two field studies conducted in flavoring production plants [ACGIH 2013].

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 2008c, d]. 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 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 meters per second [m/s]) at the face of the workstation when working with flour improvers (Figure 8-3) [Health and Safety Executive 2003i].

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.

8.2.2.1.1 Laboratory chemical hoods

Laboratory personnel will typically perform benchtop weighing and handling of flavorings in a chemical fume hood. A properly designed and maintained chemical fume hood can offer significant employee protection if used properly. There are many different hood designs, but the most common categories are the conventional or constant-flow hood, the bypass hood, and the variable air volume constant-velocity hood. The constant-flow hood is the oldest and simplest chemical hood design. The exhaust fan induces a constant volumetric airflow moving through the sash opening. For this hood design, the face velocity is lowest when the sash is wide open; when the sash is lowered the face velocity increases. The bypass hood maintains a constant hood face velocity and incorporates a bypass grille above the sash opening. When the sash is wide open it blocks the bypass grille, allowing all of the air to flow through the hood opening. As the sash is lowered, it uncovers increasingly greater amounts of the bypass grille, allowing increasing amounts of air to flow through this alternative path. If it is designed and operated properly, the amount of air flowing through the bypass grille is just sufficient to maintain a constant face velocity. Typically, however, this constant velocity can be maintained over a certain part of the sash's total range. The constant-velocity hood uses a control system to detect the sash position, face velocity and system pressure, and change the fan motor speed or other mechanism, such as mechanical dampers, to increase the airflow when the sash is raised and decrease it when the sash is lowered, thus maintaining a constant face velocity.

All chemical hoods have certain common design elements, including an exhaust fan to move air through the hood, a moving sash, exhaust slots, and a horizontal work surface (Figure 8-4). The sash can be designed to move in either a vertical or a horizontal direction. A crucial performance element for any chemical hood is the face velocity, defined as the average air velocity at the face of the hood at the sash opening. Maintaining a constant, minimum face velocity provides confidence that operations and hazardous agents within the hood will be contained. The current consensus of the literature is that the average face velocity for a laboratory chemical hood should be in the range of 80–120 fpm [Burgess et al. 2004]. The flow control system on a constant-velocity

hood should be adjusted to give a face velocity in this range. Each chemical hood should be clearly marked with the proper hood sash location that will give the desired face velocity; depending on the hood design, this could be a single location or a range of locations. Containment verification using tracer gases to provide quantitative data and smoke testing to visualize airflow patterns is recommended when the hood is installed, when substantial changes are made to the ventilation system, and periodically as part of a preventive maintenance program. In addition to the face velocity, it is important that the airflow be distributed evenly across the hood face. ANSI/AIHA Z9.5 [2003] recommends that variations of velocity across the hood face should be within ±20% of the average face velocity; however, some laboratories select a stricter standard of ±10%.

8.2.2.2 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 shown that employees who mixed butter flavorings into heated soybean oil had the highest exposures to diacetyl and the highest risk of developing severe irreversible lung disease [Kanwal et al. 2006]. These employees 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 [Kanwal et al. 2006]. NIOSH investigations at a plant where many exposed employees 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. As a result of the implementation of exposure controls, average personal diacetyl air concentrations declined in the mixing room, from 57.2 ppm to 2.88 ppm [Kanwal et al. 2011]. Exposures to diacetyl were also recorded at a plant that produced flavorings and other products in employees who added flavors to mixing and spray dryer feed tanks while the tanks were being filled. One employee 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 2009]. In addition, elevated concentrations of volatile contaminants were measured as an employee poured diacetyl-containing starter distillate from a collection vessel into a bulk container.

The use of controls to reduce employee exposure during pouring and mixing of ingredients in a commercial mixer has been evaluated in a flavoring production plant [NIOSH 2008d]. The implementation 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 an employee during the mixing of a food flavoring (Figure 8-5) [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. However, 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 HSE to contain vapors during the mixing of liquids with other liquids or solids [Health and Safety Executive 2003e]. A NIOSH laboratory study of different mixing tank hood designs for a 4 foot diameter tank showed that capture efficiencies above 90% were possible for all hoods and configurations at an exhaust flow rate of 200 cubic feet per minute (cfm) with a crossdraft of 100 fpm or less [Hirst et al. 2014].

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 compounds in the tank are captured and exhausted (Figure 8-6). 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 8-7) [Health and Safety Executive 2003a]. 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 employees as they add ingredients or operate the mixer (Figure 8-8) [ACGIH 2013; Health and Safety Executive 2003f]. 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].

8.2.2.3 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 employees in the production of flavorings, dairy products, snack foods, and in baked goods. Typically, an employee cuts open bags of material (e.g., 50-pound 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 employee exposure to the powdered flavoring; a case of bronchiolitis obliterans organizing pneumonia 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 and Darcey 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 employees as they open and dump bags of powdery materials. The designs for these devices are available from several sources of industrial ventilation guidance. The 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 8-9) [Health and Safety Executive 2003g].

Research into the effectiveness of these types of devices has shown that they can effectively reduce employee exposure to dust and vapors. A review of commercially available units showed that their use controlled dust levels to 1–2 mg/m^3 [Heitbrink and McKinnery 1986]. However, dust contamination on the surface of the bag and handling or disposal of bags caused increased employee exposure. An integral pass through to a bag disposal chute or 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 employee 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 2013].

8.2.2.4 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 compounds. 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 8-10) [Health and Safety Executive 2003b, c]. 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. The use of a pump to move flavoring compounds or finished flavorings for packaging may provide a preferable "closed transfer" approach [Health and Safety Executive 2003b]. 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 (Figure 8-11) [ACGIH 2013]. In all cases, grounding and bonding requirements should be met to prevent sparks and explosions when transferring flammable liquids [NFPA 2007].

8.2.2.5 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 2007, 2008a, b, 2009]. The use of a ventilated collar-type hood around the discharge point can help minimize employee exposure to dust and vapors. The 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 8-12) [Health and Safety Executive 2003d, h]. This design guidance recommends an 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 exhaust flow of 400–500 cfm for nontoxic dust or 1,000–1,500 cfm for toxic dust with a maximum inward air velocity of 500 fpm [ACGIH 2013].

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 8-13). 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 with 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 effective alternatives to traditional local exhaust ventilation control systems for food and flavoring production.

8.2.3 Summary of Capture Efficiencies of Control Approaches

Producing flavorings and flavored foods involves a variety of steps. These processes require the handling and manipulation of flavorings and flavoring compounds, which have been shown to be a point of exposure for employees. Table 8-2 shows the capture efficiencies of those controls which have been evaluated by NIOSH in the laboratory or in flavoring manufacturing plants and discussed in this chapter. These controls have shown to be effective at reducing potential employee exposure by 90% or greater across the wide range of processes and tasks commonly seen in flavoring and flavored food production. However, for some tasks, this may not be enough to reach the exposure control goals. When implementing engineering controls, it

Table 8-2. Summary of efficiencies for controls evaluated for the mixing of food flavorings by NIOSH

Process	Control	Evaluation	% Reduction (vs. no control)	Source
Benchtop Weighing and Handling	Slotted exhaust hood/ workstation enclosure	Simulated mixing/weighing with alcohol	97	NIOSH 2008d
	Slotted exhaust hood/ worktable	Simulated mixing/weighing with alcohol	89–100	NIOSH 2008c, Dunn et al. 2008
Bag Dumping/ Emptying	Bag dump/slotted exhaust around perimeter	Dumping of 50 lb dextrose bags	96	NIOSH 2008d
Bag Filling	Simple exterior exhaust hood	Discharge of dextrose from blender into 15 gallon containers	97	NIOSH 2008d
	Simple exterior exhaust hood	Scooping/packaging of dextrose into 15 gallon containers	64	NIOSH 2008d
Charging/ Filling Tanks and Mixers	Ventilated tank lid	Preparation of a food flavor in a large mixing tank	76	NIOSH 2008d
	Dome hood–1.5 inch gap/200 cfm EX/100 fpm CD	Tracer gas emission from mixing tank	99	Hirst et al. 2014
	Ventilated hinged lid/200 cfm EX/100 fpm CD	Tracer gas emission from mixing tank	98	Hirst et al. 2014
	Slot hood open/200 cfm EX/100 fpm CD	Tracer gas emission from mixing tank	96	Hirst et al. 2014
	Slotted back draft booth	Tracer gas emission from mixing tank	97–98	NIOSH 2008c, Dunn et al. 2008

CD = crossdraft

EX = exhaust flow rate

is important to use a tiered approach, which includes reducing the emissions at the source through containment, process modifications, or local exhaust ventilation as well as using facility provisions such as pressurization schemes. These approaches should be used in conjunction with those described below including administrative controls and the use of personal

protective equipment.

8.3 Administrative Controls

Work practices, an administrative control, are procedures followed by employers and employees 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 compounds 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 compounds should be covered when not in use. This practice will minimize the evaporation of chemicals into the workplace air and minimize likelihood of inadvertent spills. Manual handling of chemicals also provides a potentially significant source of employee 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 employee 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 PPE 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 high-efficiency particulate air (HEPA)-filtered vacuums, wet sweeping, or a properly enclosed wet vacuum system for cleaning up dust that contains diacetyl or 2,3-pentanedione. Dust should be treated as dust containing diacetyl and not as nuisance dust.
- 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 compounds, 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:

- Avoid open pouring, measuring, and transfer of diacetyl, 2,3-pentanedione, and other flavoring compounds on the FEMA priority list whenever possible [FEMA 2012].

- 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 compounds/ingredients sealed at all times.
- Maintain and use volatile flavoring compounds at the lowest possible temperature within the manufacturers' recommended temperature range for each chemical to minimize volatility.
- Use appropriate personal protective equipment during cleaning of diacetyl-containing vessels.

Some manufacturing processes may be enclosed to keep airborne diacetyl, 2,3-pentanedione, and other priority flavoring compounds contained and separated from employees 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 employees take special precautions and if necessary use appropriate PPE on entry into production work areas where diacetyl, 2,3-pentanedione, and other flavoring compounds are handled

When production processes that utilize flavorings or flavoring compounds are not enclosed or contained, employees 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 compounds, but also exposure from ingestion and dermal absorption. Important hygiene considerations include:

- Employers should not allow employees to smoke, eat, or drink in work areas where diacetyl, 2,3-pentanedione, and other flavoring compounds are used.
- Employers should provide appropriate PPE to protect the employees from dermal exposure during normal work activities. Examples include gloves, chemical resistant arm sleeves, and aprons.
- Employees should wash their hands and exposed skin before eating, drinking, or smoking.

8.3.4 Reduced Process Temperatures for Priority Flavoring Compounds

To minimize volatilization, the temperature of diacetyl, 2,3-pentanedione, and other flavoring compounds in heated tanks should be maintained as low as production processes will allow, even when closed systems are used. 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.
- Employees 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. Employees

who are involved in cleaning or are working nearby should use appropriate PPE including respiratory protection, eye, and skin protection.

8.3.6 Limit Access to Priority Flavoring Compounds

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

8.3.7 Informing Employees about the Hazard

8.3.7.1 Safety and health programs

Employers should establish a comprehensive safety and health program for all employees who are performing any activity, such as manufacturing, using, handling, or disposing of diacetyl or 2,3-pentanedione, that involves exposure to these compounds or mixtures that include these compounds. This program should include training on workplace hazards, monitoring of airborne diacetyl and 2,3-pentanedione levels, and medical surveillance of employees exposed to these compounds or mixtures that include these compounds. 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 1913 [29 CFR 1910.1200 (b)(5)].

Employee training should include information outlined in the OSHA HCS in the section titled "Employee Information and Training" [29 CFR 1910.1200 (h)(3)]. This includes information about diacetyl and 2,3-pentanedione and mixtures containing these compounds to which employees are exposed, explanation of safety data sheets and label elements, appropriate routine and emergency handling procedures, and recognition of the adverse health effects of exposure to these compounds, as well as other training requirements outlined in the OSHA HCS.

OSHA revised the HCS to align with the United Nations Globally Harmonized System of Classification and Labeling of Chemicals (GHS) in March 2012. This revision provides detailed criteria for hazard classification as well as new label elements (pictograms, signal words, hazard statements, and precautionary statements) and establishes a standardized safety data sheet (SDS) format. An SDS (formerly known as a material safety data sheet or MSDS) is a form that communicates the dangers of hazardous chemicals and mixtures and guidance for safe use. As of June 1, 2015, OSHA will require that SDSs adhere to a uniform format and include 16 sections that require specific information for the chemical or mixture listed on the SDS. More information on SDSs can be found on the OSHA HCS website at https://www.osha.gov/dsg/hazcom/index.html. Employers should be aware of the changes, requirements, phase-in dates, and compliance effective dates of the revised HCS standard. OSHA has provided additional information on the phase-in requirements and dates for the transition to the revised HCS on their website at http://www.osha.gov/dsg/hazcom/index.html.

8.3.7.2 GHS classifications of diacetyl and 2,3-pentanedione

NIOSH has provided the following classification and labeling recommendations for diacetyl (Table 8-3) and 2,3-pentanedione (Table 8-4) according to the hazard classification and labeling elements outlined in the OSHA hazard communication standard [29 CFR 1910.1200]. These classifications are based on the health hazard criteria presented in Appendix A, and

Table 8-3. Hazard classifications of diacetyl

Health hazards*					
GHS endpoint	**Hazard category**	**Rationale [reference]**	**Pictogram**	**Hazard phrase**	**Signal word†**
Acute toxicity	Category 2, inhalation	Estimated 4 hr LC50 is 441 ppm based on 6 hr exposure of 294.6 ppm in rats. Further explanation of this adjustment is provided in section 8.3.7.2.1 [Hubbs et al. 2008]		Fatal if inhaled	Danger
Serious eye damage/ eye irritation	Category 1, serious eye damage	0.1 ml of diacetyl in rabbits produced severe eye irritation with non-reversible effects after 21 days [Sugai et al. 1990]		Causes serious eye damage	Danger
Skin sensitization	Category 1B, skin sensitizer	EC3 values ranged from 11.3%–17.9% in mice via local lymph node assay [Anderson et al. 2011; Anderson et al. 2013; Roberts et al. 1999]		May cause an allergic skin reaction	Warning
Specific target organ toxicity-single exposure‡	Category 1	Epithelial necrosis and inflammation in the trachea and larynx in rats at 224 ppm [Hubbs et al. 2008]		Causes damage to the respiratory system if inhaled	Danger
Specific target organ toxicity- repeated exposure‡	Category 1	Peribronchial lymphocytic infiltrates in mice at 25 ppm [Morgan et al. 2008; National Toxicology Program 2011]. Several case studies, public health investigations, and a cohort mortality follow-up study link exposure to flavorings containing diacetyl to fixed airway obstruction [Akpinar-Elci et al. 2004; Cavalcanti et al. 2012; CDC 2002, 2007, 2013; Halldin et al. 2013].		Causes damage to respiratory system through prolonged or repeated exposure if inhaled	Danger

See footnotes at end of table.

(Continued)

Table 8-3 (Continued). Hazard classifications of diacetyl

Health hazards*					
GHS endpoint	**Hazard category**	**Rationale [reference]**	**Pictogram**	**Hazard phrase**	**Signal word†**
Physical hazards*					
Flammable liquid	Category 2	6°C [IPCS 2009]; 7°C [Sigma Aldrich 2010]		Highly flammable liquid and vapor	Danger

*Precautionary statements for the health and physical hazard classifications presented can be found in Appendix C of the hazard communication standard [29 CFR 1910.1200].

†Appendix C of the hazard communication standard [29 CFR 1910.1200] provides several precedence rules regarding the application of pictograms and signal words as well as rules for combining or omitting hazard and precautionary statements. These precedence rules save space on the label and improve readability.

‡NIOSH recommends that these GHS classifications should appear on product labels and SDSs when found in mixtures below the specific cut-off values/concentration limits that are provided in the hazard communication standard [29 CFR 1910.1200]. See section 8.3.7.3 below for further information.

Table 8-4. Hazard classifications of 2,3-pentanedione

Health hazards*					
GHS endpoint	**Hazard category**	**Rationale [reference]**	**Pictogram**	**Hazard phrase**	**Signal word†**
Acute toxicity	Category 2, inhalation	Estimated 4 hr LC50 is 441 ppm based on 6 hr exposure of 294.6 ppm. Further explanation of this adjustment is provided in section 8.3.7.2.1 [Hubbs et al. 2012; Morgan et al. 2012]		Fatal if inhaled	Danger
Skin sensitization	Category 1B, skin sensitizer	EC3 value 15.4 % in mice via local lymph node assays		May cause an allergic skin reaction	Warning
Specific target organ toxicity-single exposure‡	Category 1	6-hour inhalation study in rats at 112 ppm caused necrotizing rhinitis. At 120 ppm exposure for 6 hrs, decreased airway activity to methacholine aerosol was observed. [Hubbs et al. 2012]. After 120, 240, and 320 ppm exposure for 6 hr, decreased airway activity to methacholine aerosol was observed [Zaccone et al. 2013].		Causes damage to the respiratory system if inhaled	Danger
Specific target organ toxicity-repeated exposure‡	Category 1	Inhalation exposure study (6 hours/day, 5 days/week, for 2 weeks) in rats caused damage to airway epithelium, potential disruption to underlying basement membrane, and statistically significant influx of neutrophils in BALF at 200 ppm. In mice, the same repeat dose exposure regimen caused nasal turbinate necrosis (100 ppm) and laryngeal lesions (50 ppm). [Morgan et al. 2012]		Causes damage to respiratory system through prolonged or repeated exposure if inhaled	Danger

See footnotes at end of table.

(Continued)

Table 8-4 (Continued). Hazard classifications of 2,3-pentanedione

Health hazards*					
GHS endpoint	**Hazard category**	**Rationale [reference]**	**Pictogram**	**Hazard phrase**	**Signal word†**
Physical hazards					
Flammable liquid	Category 2	[Chem Service Inc. 1988; Merck Chemicals International 2010]		Highly flammable liquid and vapor	Danger

*Precautionary statements for the health and physical hazard classifications presented can be found in Appendix C of the hazard communication standard [29 CFR 1910.1200].

*Precautionary statements for the health and physical hazard classifications presented can be found in Appendix C of the hazard communication standard [29 CFR 1910.1200].

†Appendix C of the hazard communication standard [29 CFR 1910.1200] provides several precedence rules regarding the application of pictograms and signal words as well as rules for combining or omitting hazard and precautionary statements. These precedence rules save space on the label and improve readability.

‡NIOSH recommends that these GHS classifications should appear on product labels and SDSs when found in mixtures below the specific cut-off values/concentration limits that are provided in the hazard communication standard [29 CFR 1910.1200]. See section 8.3.7.3 below for further information.

physical hazard criteria presented in Appendix B of the hazard communication standard [29 CFR 1910.1200]. These classifications are based on the data from employee investigations (Chapter 3) and from experimental toxicology studies (Chapter 4). OSHA has provided guidance on hazard communication for diacetyl and food flavorings that contain diacetyl [OSHA 2013] on the basis of the previous version of the HCS, but that guidance does not address some of the requirements in the revised HCS based on GHS.

8.3.7.2.1 Further justification of acute inhalation toxicity for diacetyl and 2,3-pentanedione

The GHS classification for acute inhalation toxicity, category 2 for diacetyl is based upon rat acute inhalation studies of diacetyl and 2,3-pentanedione [Hubbs et al. 2012; Hubbs et al. 2008]. In the diacetyl study, the histopathology changes seen in rats exposed for 6 hours to a time-weighted average of 294.6 to 365 ppm diacetyl would be predicted to cause death if the animals had been observed for a longer time period. In exposures conducted in this concentration range, the severity scores in the airway epithelium of trachea, larynx, and multiple sections of nose had an average score of 7.5 to 9.5 on a scale of 1 to 10 (with 10 being most severe). Damage to airway epithelium is the accepted underlying cause for obliterative bronchiolitis in man, which causes human morbidity and mortality [King 1989]. The importance of extrapulmonary airway injury in the rodents to human risk assessment is discussed in the toxicology section.

In the 2,3-pentanedione inhalation study in rats, clinical observations documented that no clinical signs were present immediately after the 6 hour inhalation exposures to 318 or 354 ppm but respiratory signs were present in more than half of the rats at 18 hours post-exposure, when the rats were sacrificed [Hubbs et al. 2012].

While both of these inhalation studies were not intended to produce lethality, contemporary laboratory animal studies frequently use early indicators of impending mortality rather than actual mortality for studies of lethality [Stokes 2002]. The presence of extensive respiratory epithelial damage in 100% of the rats at exposures of approximately 294.6 ppm or greater for 6 hours in both of these studies and time-dependent progressive respiratory clinical signs are considered a humane endpoint for use in place of mortality. In this case, expert scientific judgment needs to be used to determine the LC50 because of the humane considerations. Because all rats had high pathology scores after inhaling 294.6 ppm or higher, NIOSH concludes that the LC50 based on a 4-hour exposure would be 441 ppm (the 4-hr equivalent of 294.6 ppm) or less. After inhaling 100 to 120 ppm diacetyl for 6 hours, histopathology changes were limited to the first nasal section and single exposures at this concentration did not suggest potential acute lethality. Similarly, after inhaling 111 ppm 2,3-pentanedione for 6 hours, rats did not have clinical signs and significant histopathology changes were limited to the first two nasal sections. This equates to a GHS acute inhalation toxicity category 2 classification (>100 and <500 ppm) for both diacetyl and 2,3-pentanedione.

8.3.7.3 Classifying mixtures containing diacetyl and 2,3-pentanedione

The HCS indicates that mixtures that contain compounds that require classification and labeling can be evaluated under a set of bridging principles if no toxicological data are available for the mixture itself. These bridging principles can be applied when there is "sufficient data on both the individual ingredients and similarly tested mixtures to adequately characterize the hazards of the mixture" [29

CFR 1910.1200.A.0.5]. If these bridging principles cannot be applied, the HCS provides specific cut-off values/concentration limits that are specified for each health hazard class and category. Most of these specific cut-off values/concentration limits are either ≥0.1% or ≥1%, under which mixtures containing classified compounds should be labeled accordingly. However, a few endpoints have different specific cut-off value/concentration limits specified. For most of the chemical hazards for which NIOSH made classifications (Tables 8-3 and 8-4), the specific cut-off values/concentration limits specified by the HCS are ≥1%. Exceptions include the hazard category for "serious eye damage/eye irritation" (≥3%) and for "flammable liquids," for which the HCS does not have a cut-off value/concentration limit. If these mixtures contain classified compounds below the specified HCS cut-off values/concentration limits, classification and labeling of those mixtures are not usually required. However, the standard indicates that "while the adopted cut-off values/concentration limits adequately identify the hazard for most mixtures, there may be some that contain hazardous ingredients at lower concentrations than the specified cut-off values/concentration limits that still pose an identifiable hazard [29 CFR 1910.1200.A.0.4.3.1]. As explained below, this is an important consideration for mixtures containing diacetyl and 2,3-pentanedione.

Cal/OSHA provided industrial hygiene monitoring results from a Flavor Industry Safety and Health Evaluation Program evaluation in 2006 and 2007 at a food flavoring manufacturer for the production of vanilla dry blend product [Widess 2013]. In this evaluation, a task-based personal breathing zone sample concentration of diacetyl collected over 19 minutes ranged from 3.5 to 5 ppm during dispensing of dry powder containing 0.14% diacetyl by weight. If a TWA exposure was calculated over an 8-hour work shift, assuming no other diacetyl exposure during the work shift, the 8-hour TWA exposure would have been 0.2 ppm, which exceeds the NIOSH 8-hour TWA REL (0.005 ppm). The exposure in the Flavor Industry Safety and Health Evaluation Program evaluation also exceeds the NIOSH STEL for diacetyl (0.025 ppm). In a NIOSH evaluation at a wholesale flavors and colors manufacturer, a task sample was collected when an employee was packaging dairy based flavoring into small containers over 33 minutes. Diacetyl comprised less than 1% of the total dairy flavored powder formulation. If a TWA exposure was calculated over an 8-hour work shift, assuming no other diacetyl exposure, the 8-hour TWA exposure would have been 0.33 ppm, which also exceeds the NIOSH 8-hour TWA REL [NIOSH 2008a]. Additionally, a laboratory-based study also identified emissions of diacetyl from natural butter and butter flavor powders, pastes, and liquid products in a laboratory environment [Rigler and Longo 2010]. Determinations show that even in the butter flavoring containing the lowest amount of diacetyl in the bulk flavoring (1.01% by weight), heating this flavoring to 37.5°C released vapor concentrations of diacetyl as high as 13.67 ppm. This suggests that even if diacetyl is present in bulk concentrations of <1%, vapor concentrations of diacetyl could greatly exceed the NIOSH REL and STEL. NIOSH does not have data to confirm this same relationship between concentrations in bulk mixture and air for 2,3-pentanedione. Although the vapor pressure of 2,3-pentanedione (21.4 mm Hg at 20°C) is lower than diacetyl (52.2 mm Hg at 20°C) and will not volatilize as readily as diacetyl at room temperature, the initial boiling point of 2,3-pentanedione (108°C) suggests that it is still a volatile organic compound [EPA 2013] that can readily enter the vapor phase upon heating, leading to employee exposures.

The data presented in this criteria document strongly suggest that diacetyl and

2,3-pentanedione are toxic to the respiratory system at very low vapor concentrations. For this reason, NIOSH recommends that flavoring mixtures that contain diacetyl or 2,3-pentanedione should be provided on product labels and SDSs at concentrations below the default GHS mixture cutoff points. Specifically, NIOSH recommends labeling at concentrations that under the anticipated conditions of use could generate vapors exceeding the NIOSH REL and/or STEL. In these cases the labels and SDSs should carry the pictogram, hazard phrase, signal word, and precautionary statements for the *specific target organ toxicity-single exposure* and *specific target organ toxicity-repeated exposure* endpoints. If specific cut-off values can be established otherwise, this recommendation does not need to be followed.

Regarding the nonrespiratory endpoints under which diacetyl and 2,3-pentanedione have been classified by NIOSH (Tables 8-3 and 8-4), NIOSH does not have any data to suggest that mixtures containing these compounds in concentrations less than the specific cutoff values/concentration limits specified by the HCS are hazardous. This includes the *acute toxicity, skin corrosion/irritation, serious eye damage/eye irritation, skin sensitization, and flammable liquid* endpoints for diacetyl, and *acute toxicity and flammable liquid* endpoints for 2,3-pentanedione. NIOSH recommends that manufacturers carefully evaluate whether mixtures containing these compounds below the cut-off values/concentration limits specified in the HCS should be labeled.

The Flavor and Extract Manufacturers Association has recommended that several flavoring substances, including diacetyl and 2,3-pentanedione, should include the following label warning if they are present in compounded flavors (including liquid and dry or powdered mixtures) in any concentration if they will be heated during processing [FEMA 2012]:

WARNING – This flavor may pose an inhalation hazard if improperly handled. Please contact your workplace safety officer before opening and handling, and read the MSDS. Handling of this flavor that results in inhalation of fumes, especially if the flavor is heated, may cause severe adverse health effects.

FEMA has also recommended that this same warning should be used for containers of neat substances such as diacetyl and 2,3-pentanedione as well as other "high priority" substances listed in the FEMA guidance document. Additionally, FEMA has recommended that all containers of compounded flavors (liquid and dry or powdered) or natural flavoring complexes that contain diacetyl, 2,3-pentanedione or other flavoring substances in concentrations of >1.0% should be labeled with the above warning [FEMA 2012]. It is of note that the use of the word "warning" in the FEMA text is inconsistent with the specific criteria for its use and application as a signal word in the HCS. NIOSH recommends removal of the word "warning" when using the FEMA text (see section 8.3.7.4 for details)

8.3.7.4 Labeling and posting

To communicate hazard information effectively to employees, employers should:

- Post appropriate labeling on all flavoring product containers according to the HCS requirements [29 CFR 1910.1200]. In this document, NIOSH is providing the recommended label elements, including signal word, hazard statements, and pictograms, that should be included for labeling of diacetyl and 2,3-pentanedione on SDSs and labels for shipping containers [see Tables 8-3 and 8-4]. The

precautionary statements that are also required can be found in Appendix C to the HCS [29 CFR 1910.1200]. NIOSH also recommends that mixtures containing diacetyl or 2,3-pentanedione at any concentration that could generate vapors that could exceed the NIOSH REL and/or STEL carry the pictogram, hazard phrase, and signal word for the *specific target organ toxicity-single exposure* and *specific target organ toxicity-repeated exposure* classifications until it can be demonstrated that mixtures containing these compounds in concentrations less than the specific cut-off values/concentration limits specified by HCS are not harmful.

- Place the following warning, as recommended by FEMA [FEMA 2012], on containers of compounded flavors that contain diacetyl, 2,3-pentanedione, or other flavoring substances identified in Table 1 of the FEMA document, in any concentration if the flavors are to be heated: *This flavor may pose an inhalation hazard if improperly handled. Please contact your workplace safety officer before opening and handling, and read the MSDS. Handling of this flavor that results in inhalation of fumes, especially if the flavor is heated, may cause severe adverse health effects.* Note: While NIOSH agrees with the content of the italicized text above, the word "warning," which appears in the FEMA guidance document, was not included because it is inconsistent with the specific criteria for its use and application as a signal word in the HCS. NIOSH recommends that the word "warning" should not be included on hazard statements containing diacetyl or 2,3-pentanedione, as this word has specific meaning and conflicts with standardized HCS signal word terminology.
- Post warning labels and signs describing the health risks associated with flavoring compound exposures at entrances to work areas and inside work areas where diacetyl, 2,3-pentanedione, or other flavoring compounds are used.
- Post warning labels and signs describing any needs for PPE in the work area.
- Post the statement "Wear Respiratory Protection in this Area" if respiratory protection is required.
- Print all labels and warning signs in English and in the predominant language of employees who do not read English.
- Verbally inform employees about the hazards and instructions printed on the labels and signs if they are unable to read them.
- Follow the requirements of the HCS for classifying and labeling diacetyl, 2,3-pentanedione, and other flavoring compounds. NIOSH recommends that development of SDSs and labels should occur as soon as possible given the importance of warning users about exposures of diacetyl and 2,3-pentanedione above the REL and or STEL. The OSHA website has additional information on the hazard communication standard at http://www.osha.gov/dsg/hazcom/index.html.

8.3.7.5 Training

Employees should receive training as mandated by the HCS [29 CFR 1910.1200]. As part of the training, employers should also:

- Inform all potentially exposed employees, including temporary and contract employees, about diacetyl or 2,3-pentanedione-associated health risks such as acute toxicity, skin irritation and sensitization, eye irritation or damage, respiratory disease, and flammability hazards.

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

8.4 Respiratory Protection

Respirators should not be used as the primary means of controlling employee exposures to inhalation hazards for routine operations. Whenever possible, engineering and work practice control techniques discussed above should be used. 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 reduce exposures to concentrations below the REL.

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 is required to implement a cartridge/canister change schedule based on objective information that will ensure that the cartridges/canisters are changed before the end of their service life, according to the OSHA respiratory protection standard which was revised in 1998. The revised OSHA respiratory protection standard removed the previous method of determining the end of a cartridge's service life by using warning properties such as odor and irritation. 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 is used to determine 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 such as odor and irritation 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. All of the air-purifying respirators listed in Table 8-5 are equipped with combination organic vapor/P100 or organic vapor/high efficiency filter cartridges, which are capable of protecting wearers against both vapor and particulate hazards.

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 2010].

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. Employee activity and employee 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 2004].

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 compounds containing diacetyl that could cause dermal injury

Table 8-5. 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)	1,000	5 ppm (5,000 ppb)	9.3 ppm (9,300 ppb)
PAPR, hood or helmet w/OV-HE cartridge(s) or canister(s)	25/1,000†	0.12/5 ppm (120/5,000 ppb)	0.23/9.3 ppm (230/9,300 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, continuous flow mode or pressure- demand mode or other positive- pressure mode, full facepiece	1,000	5 ppm (5,000 ppb)	9.3 ppm (9,300 ppb)
SAR, hood or helmet	25/1,000†	0.12/5 ppm (120/5,000 ppb)	0.23/9.3 ppm (230/9,300 ppb)
SAR, loose fitting facepiece	25	0.12 ppm (120 ppb)	0.23 ppm (230 ppb)
SCBA, pressure-demand or other positive-pressure mode (e.g. open/closed circuit), full facepiece or hood/helmet	10,000	50 ppm	93 ppm

PAPR = Powered air-purifying respirator

SAR = Supplied air respirator

OV-HE = Organic vapor-high efficiency particulate

SCBA = Self-contained breathing apparatus

*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 assigned protection factor (APF) of 1,000. Absent such evidence, these respirators receive an APF of 25.

[29 CFR 1910.138]. It is important to select the most appropriate chemical resistant glove for the application and to determine how long it can be worn, and whether it can be reused. Procedures should be implemented to ensure that the gloves are replaced before breakthrough occurs. NIOSH recommends that before purchasing gloves or other protective clothing, the employer should refer to the SDS from the manufacturer of the diacetyl and 2,3-pentanedione being used, and /or request documentation from the glove or protective clothing manufacturer that the gloves meet the appropriate test standard(s) for the hazard(s) anticipated, and to request any glove and protective clothing breakthrough time data against diacetyl and 2,3-pentanedione that may be available from these sources. 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 2010 [ANSI 2010]. 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 employees 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 Z87.1-2010 [ANSI 2010]. 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 2013]. 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.

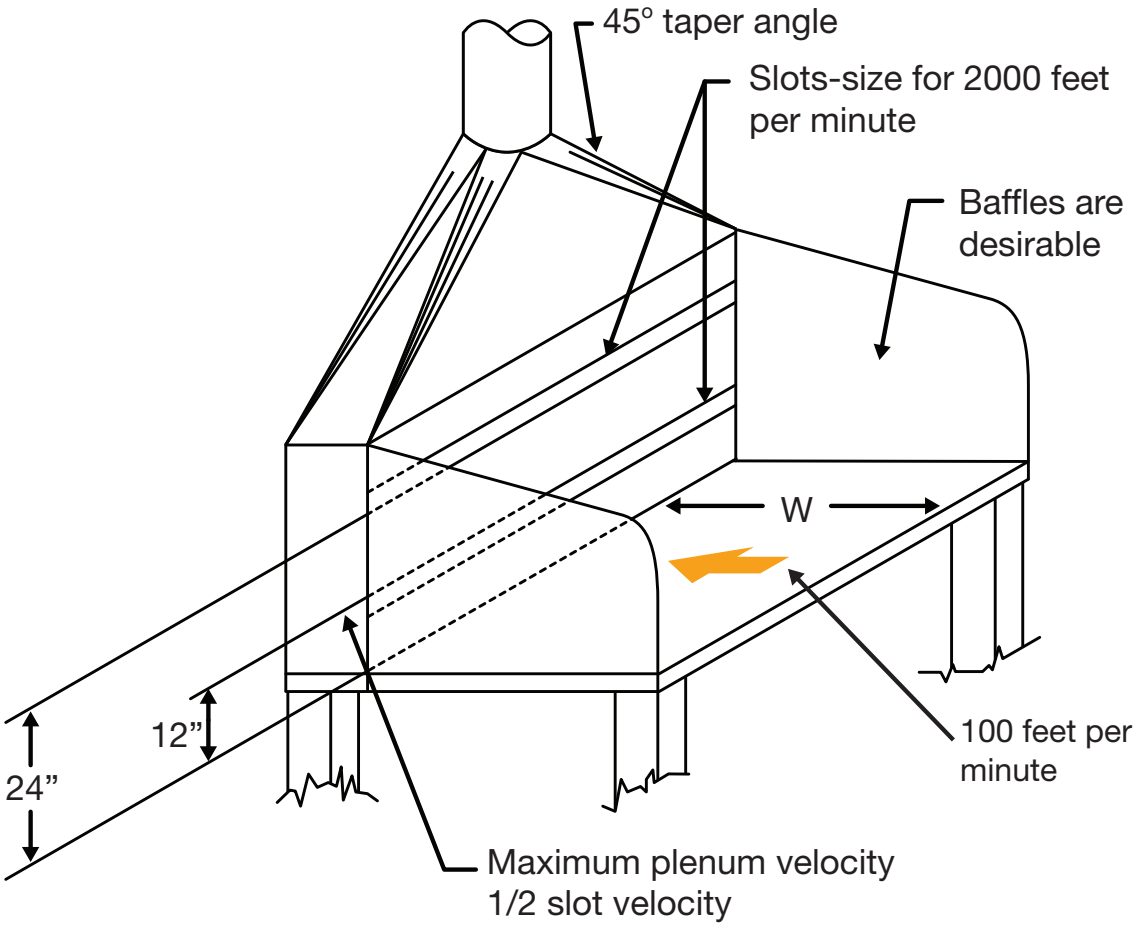


Figure 8-1. Welding ventilation bench hood*

*VS-90-01, From ACGIH, Industrial Ventilation: A Manual of Recommended Practice for Design, 26th Edition. Copyright 2009. Reprinted with permission

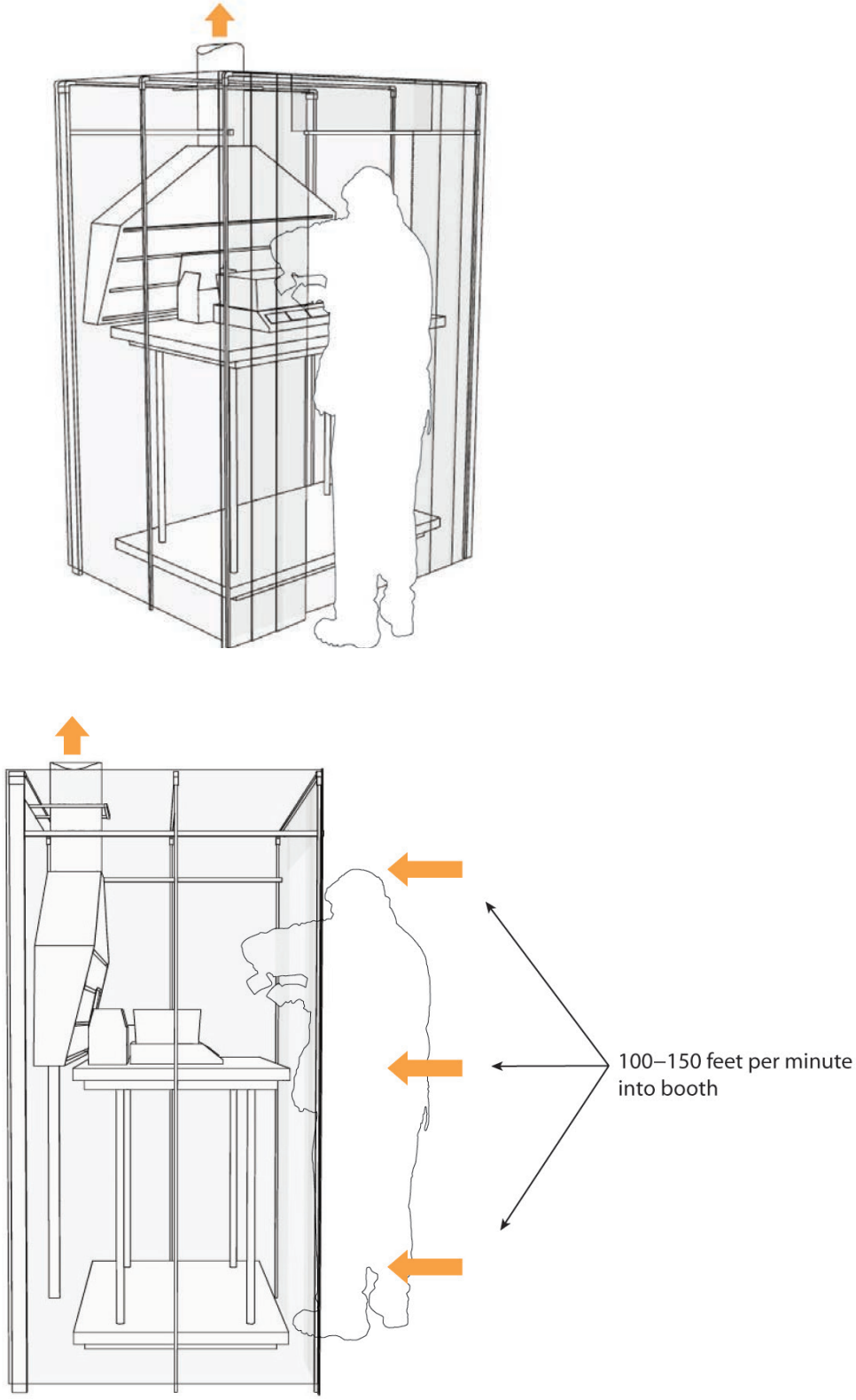


Figure 8-2. Ventilated small batch mixing workstation

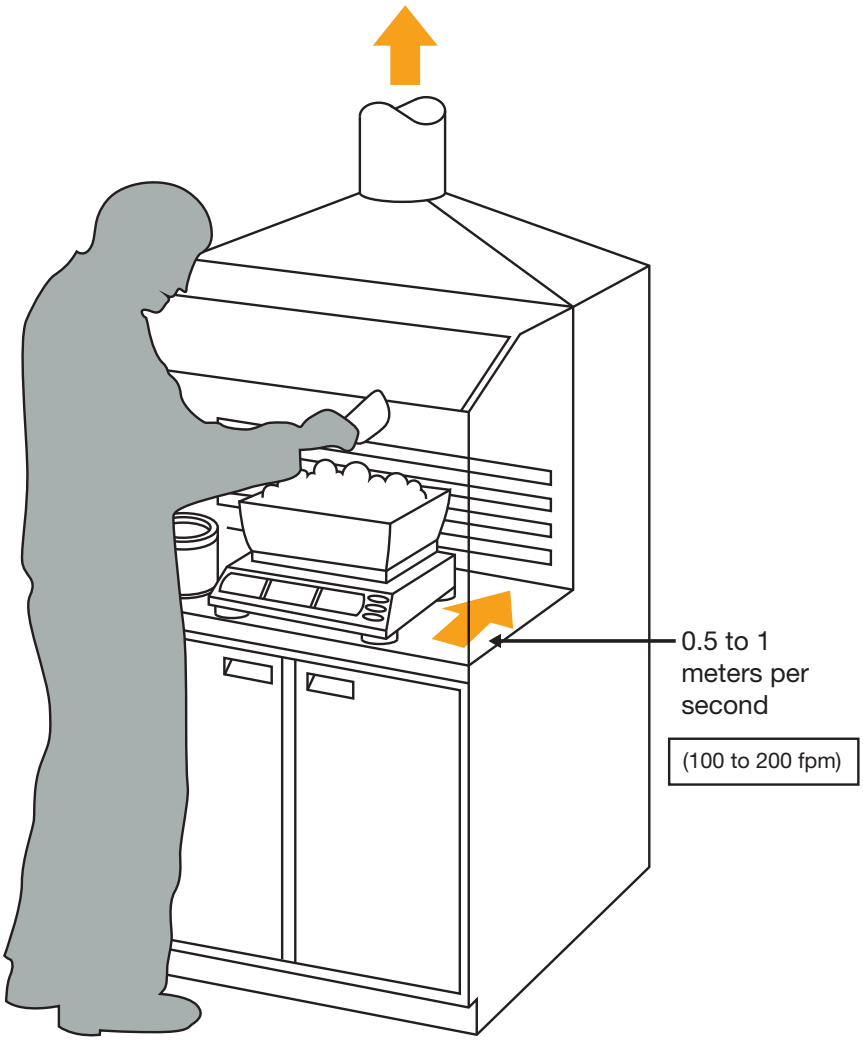


Figure 8-3. Benchtop ventilation for weighing and handling powders 0.5 to 1 m/s = 100 to 200 fpm*

*Contains public sector information published by the Health and Safety Executive and licensed under the Open Government License v1.0.

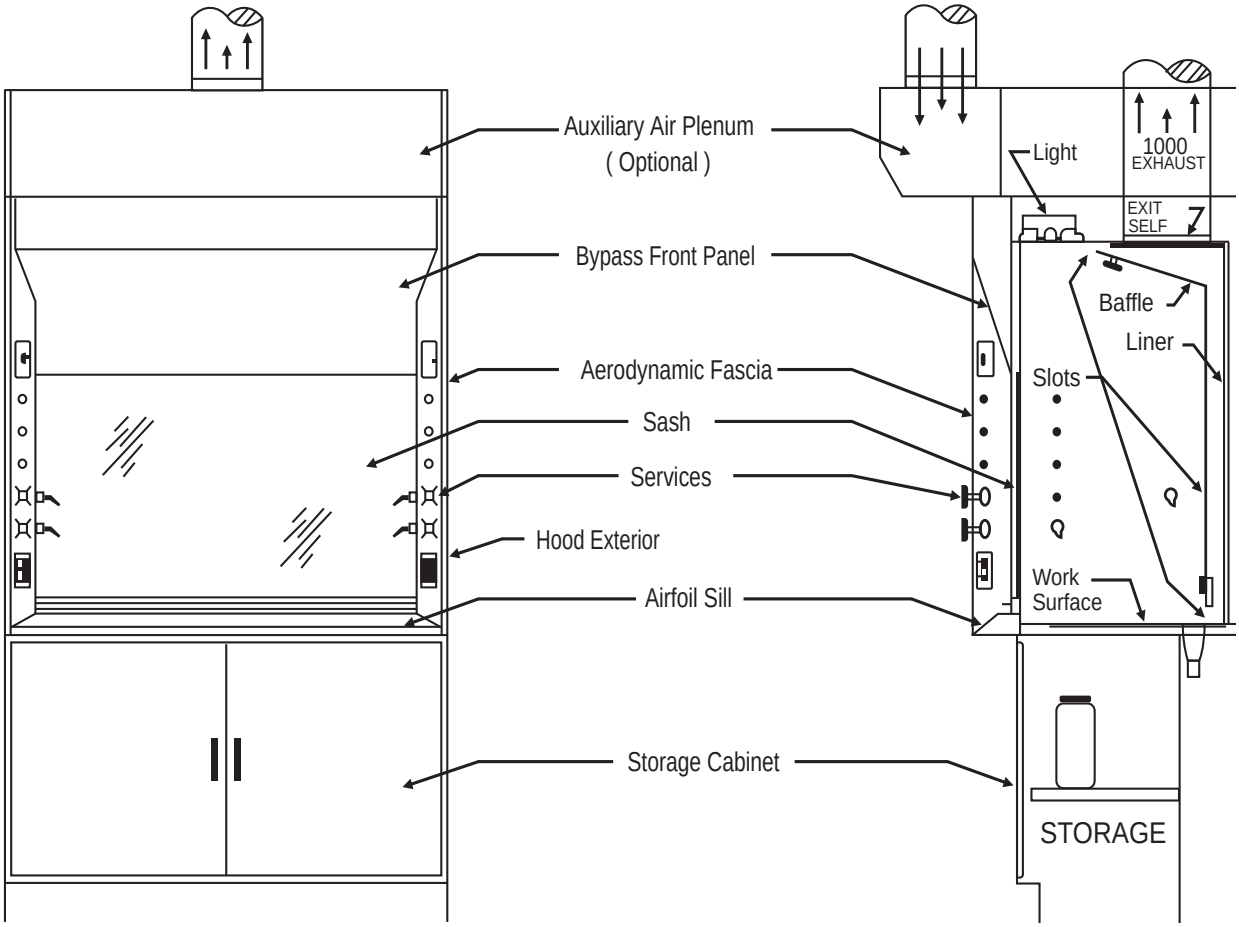


Figure 8-4. Schematic of a laboratory chemical fume hood*

*Reprinted from SEFA 1-2002, Laboratory Fume Hoods: Recommended Practices[SEFA 2002].

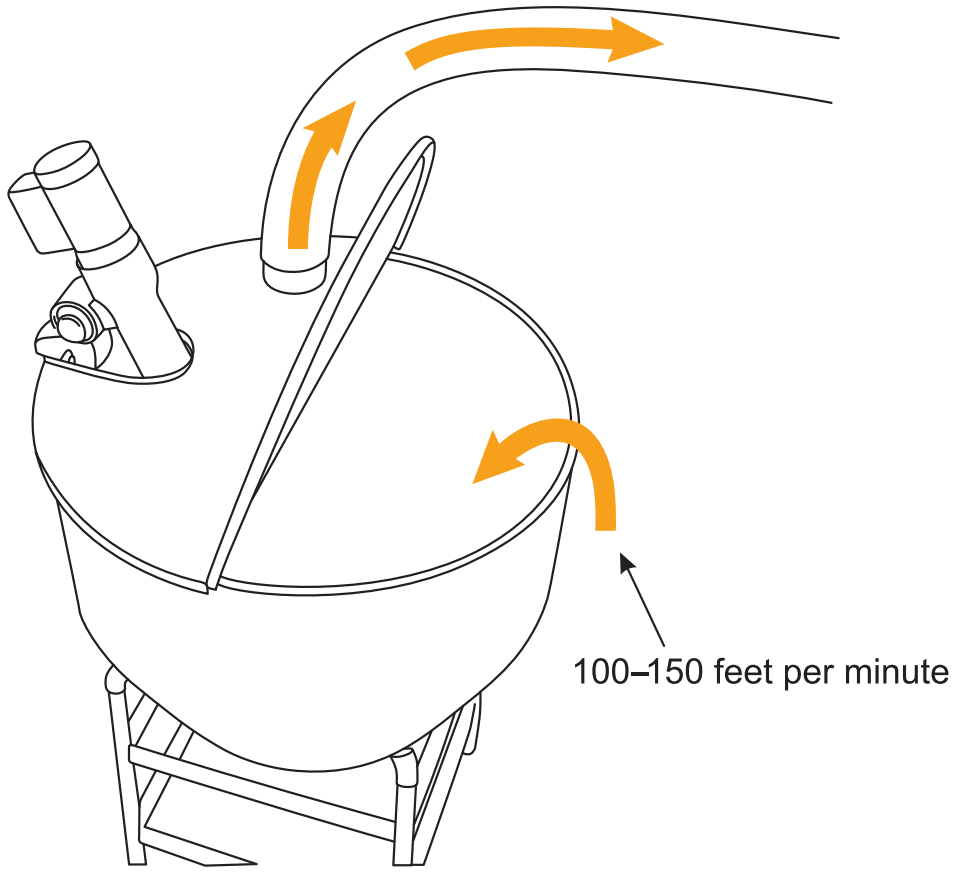


Figure 8-5. Mixing vessel with a ventilated hinged tank lid

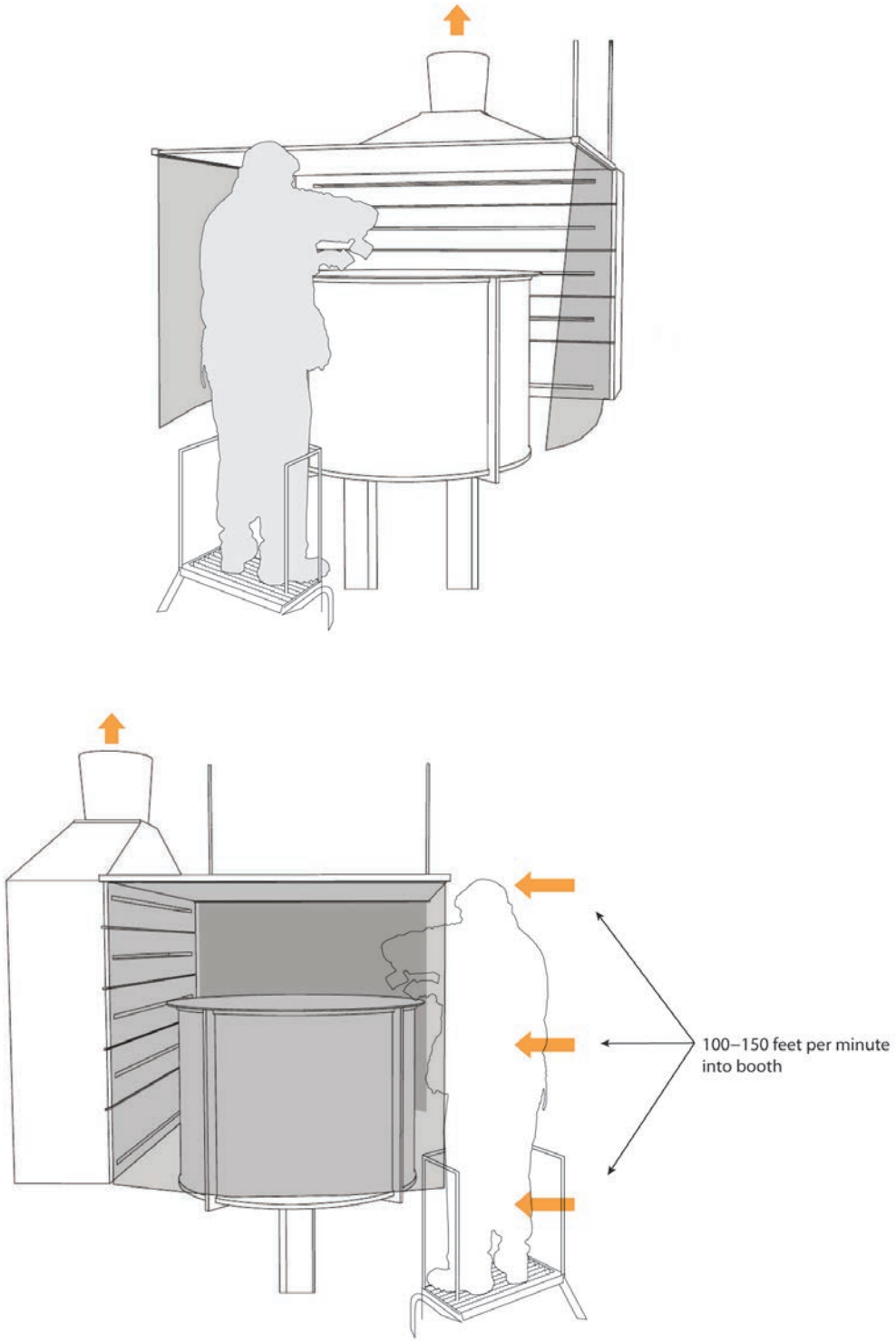


Figure 8-6. Ventilated booth for large batch mixing

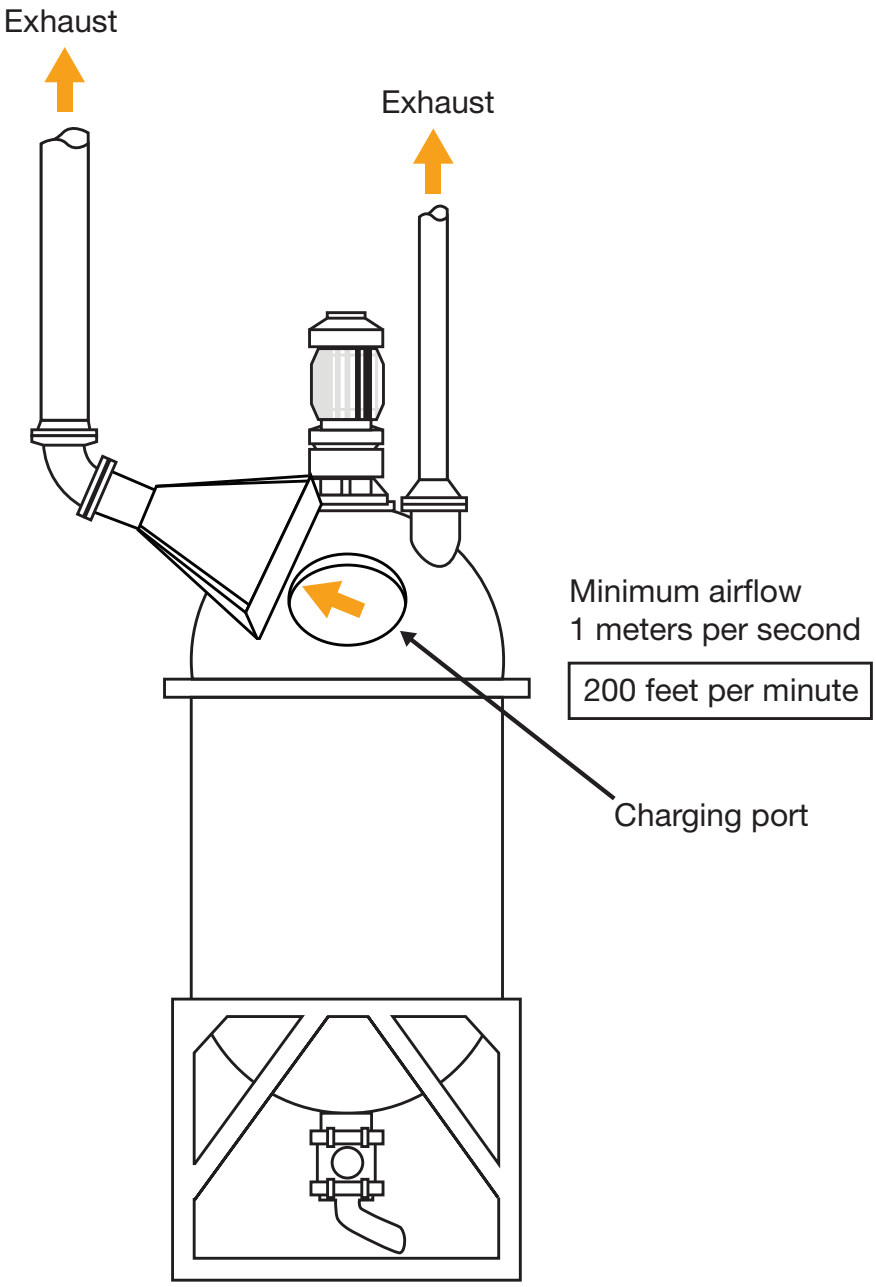


Figure 8-7. Charging reactors and mixers from a sack or keg*, 1 m/s = 200 fpm

*Contains public sector information published by the Health and Safety Executive and licensed under the Open Government License v1.0.

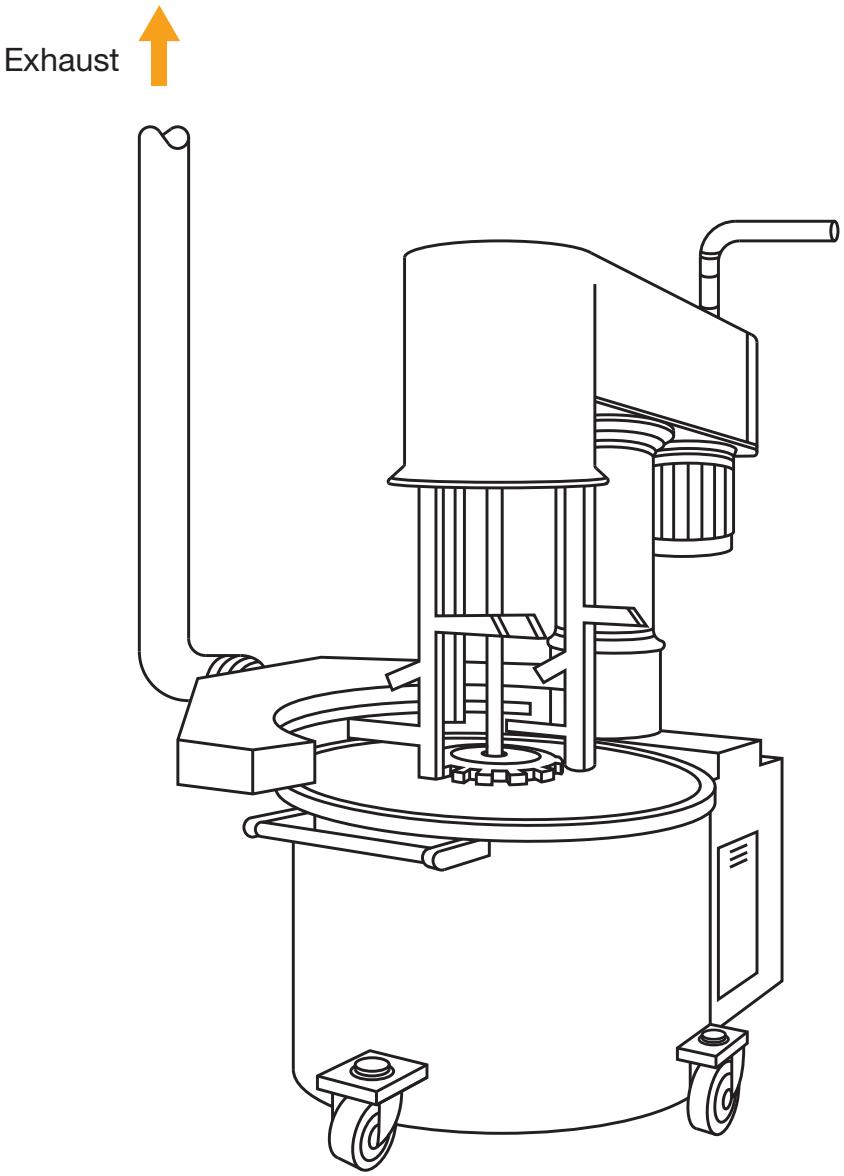


Figure 8-8. Annular exhaust for capturing dusts/vapors from mixers

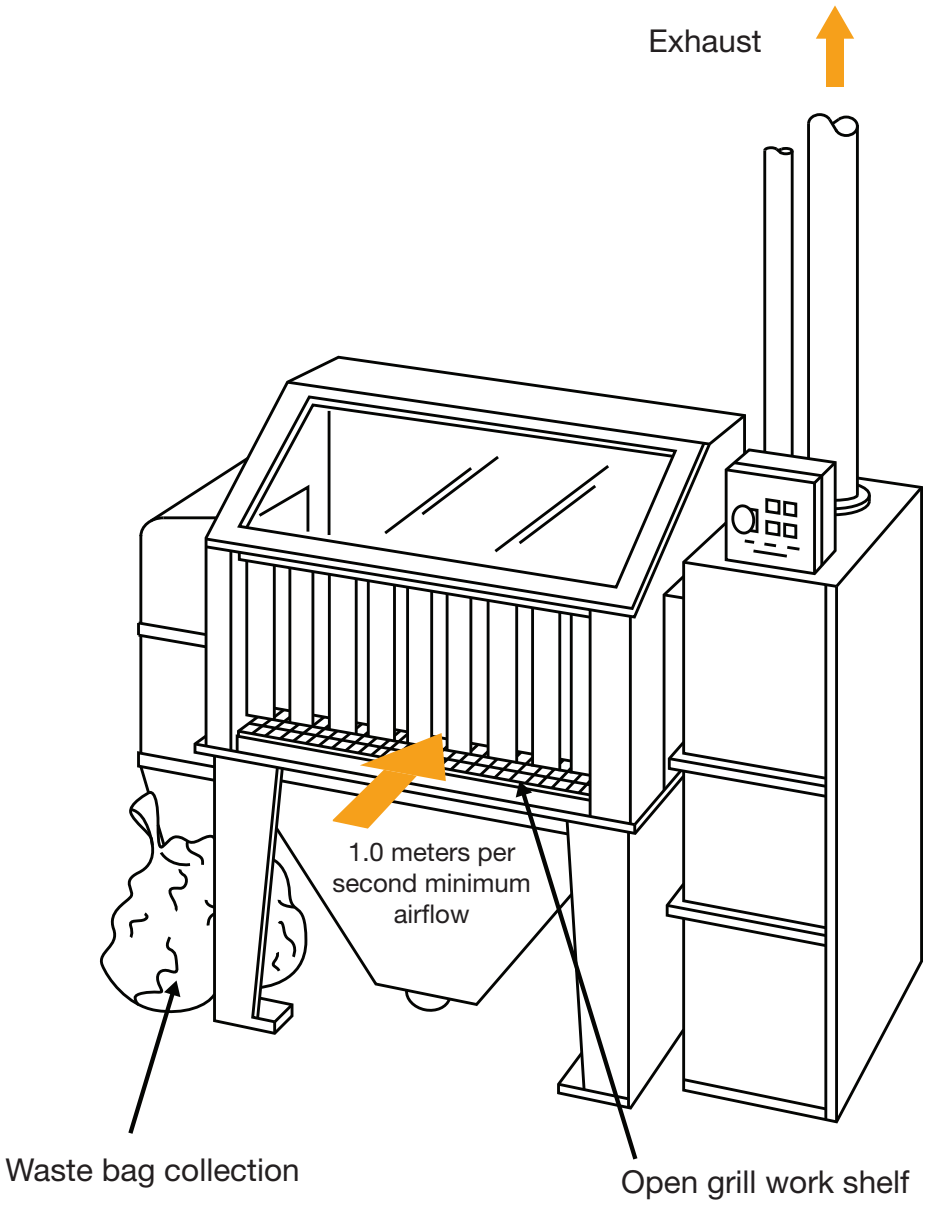


**Figure 8-9. Ventilated bag dumping/emptying station*
1.0 m/s = 200 fpm**

*Contains public sector information published by the Health and Safety Executive and licensed under the Open Government License v1.0.

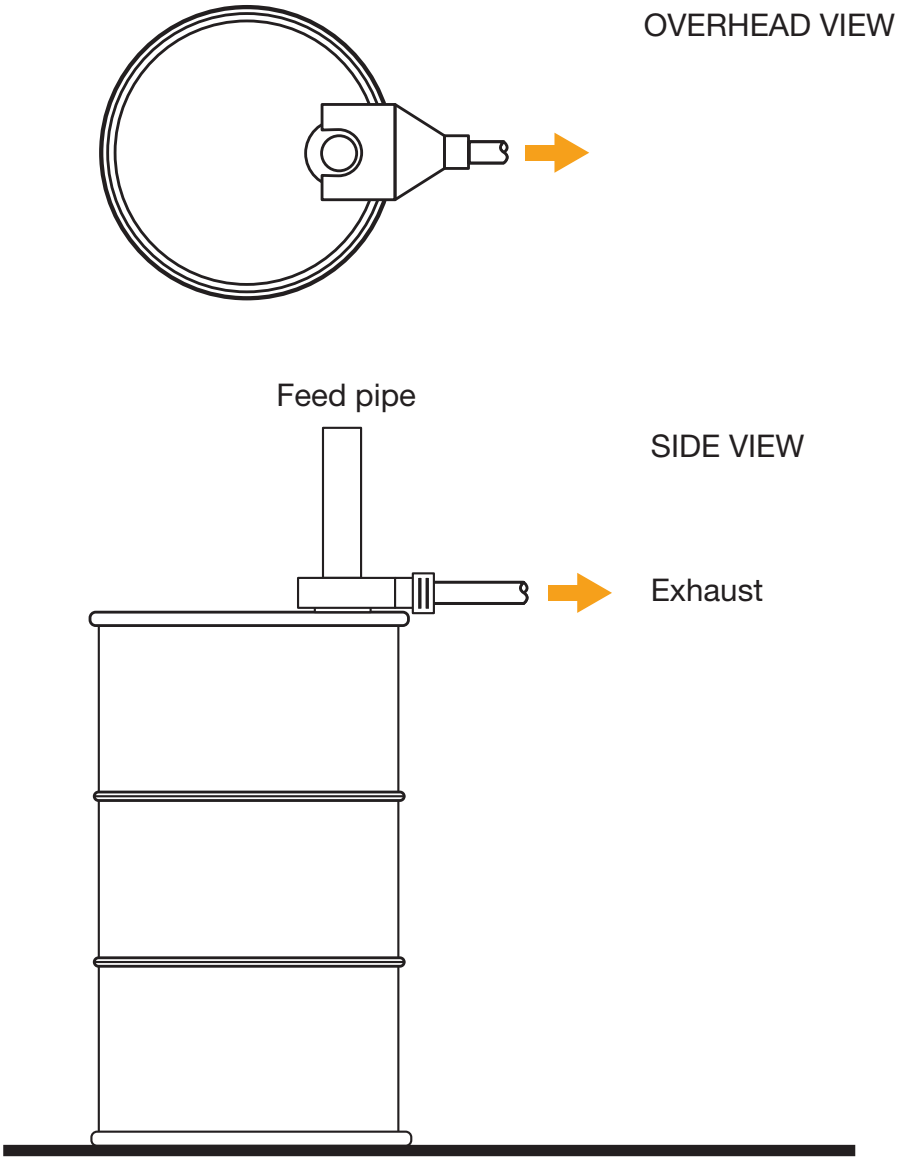
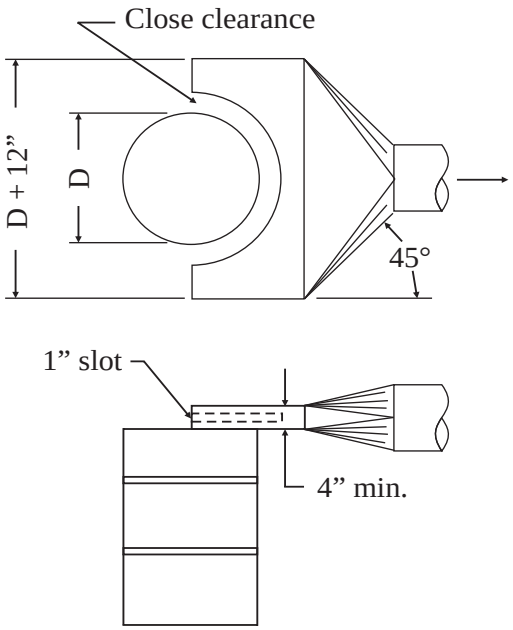
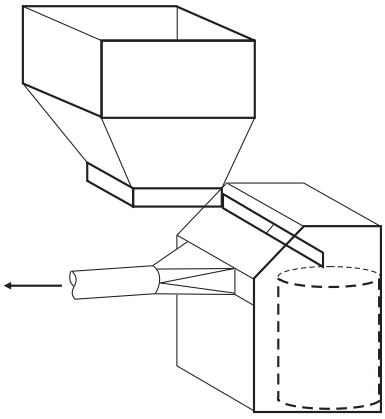


Figure 8-10. Annular exhaust for capturing vapors during drum filling*

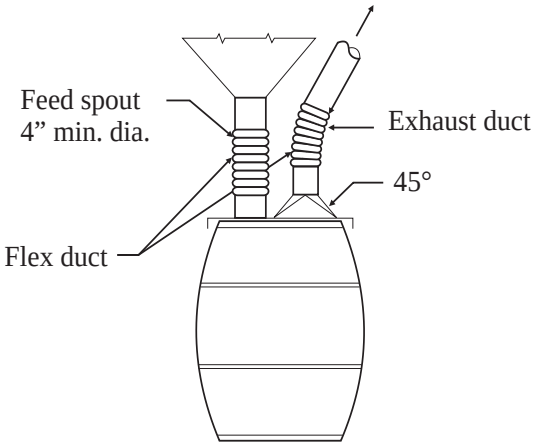
*Contains public sector information published by the Health and Safety Executive and licensed under the Open Government License v1.0



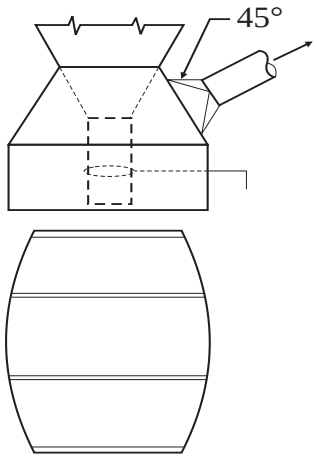
Q = 100 cfm/ft^2 barrel top (minimum)
Minimum duct velocity = 3500 fpm
h_e= 1.78 VP_s + 0.25 VP_d



Q = 150 cfm/ft^2 of open face area
Minimum duct velocity = 3500 fpm
h_e= 0.25 VP_d (45°) taper)



Q = 50 cfm × drum diam. (ft)
Minimum duct velocity = 3500 fpm
h_e= 0.25 VP_d



Q = 300–400cfm
Minimum duct velocity = 3500 fpm
h_e= 0.25 VP_d

Figure 8-11. Ventilation design options for capturing vapors during drum filling*

*VS-15-01, From ACGIH, *Industrial Ventilation: A Manual of Recommended Practice for Design*, 26th Edition. Copyright 2009. Reprinted with permission.

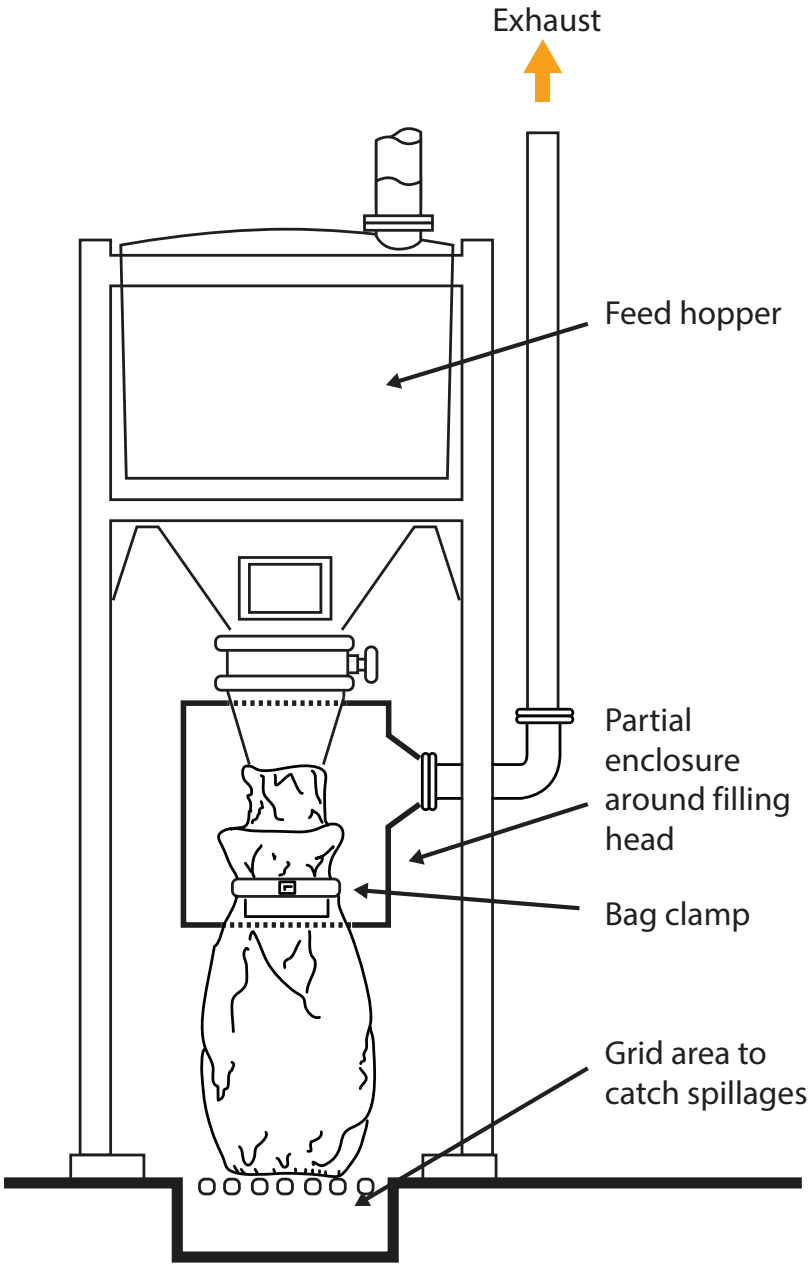


Figure 8-12. Ventilation for bag filling*

*Contains public sector information published by the Health and Safety Executive and licensed under the Open Government License v1.0.

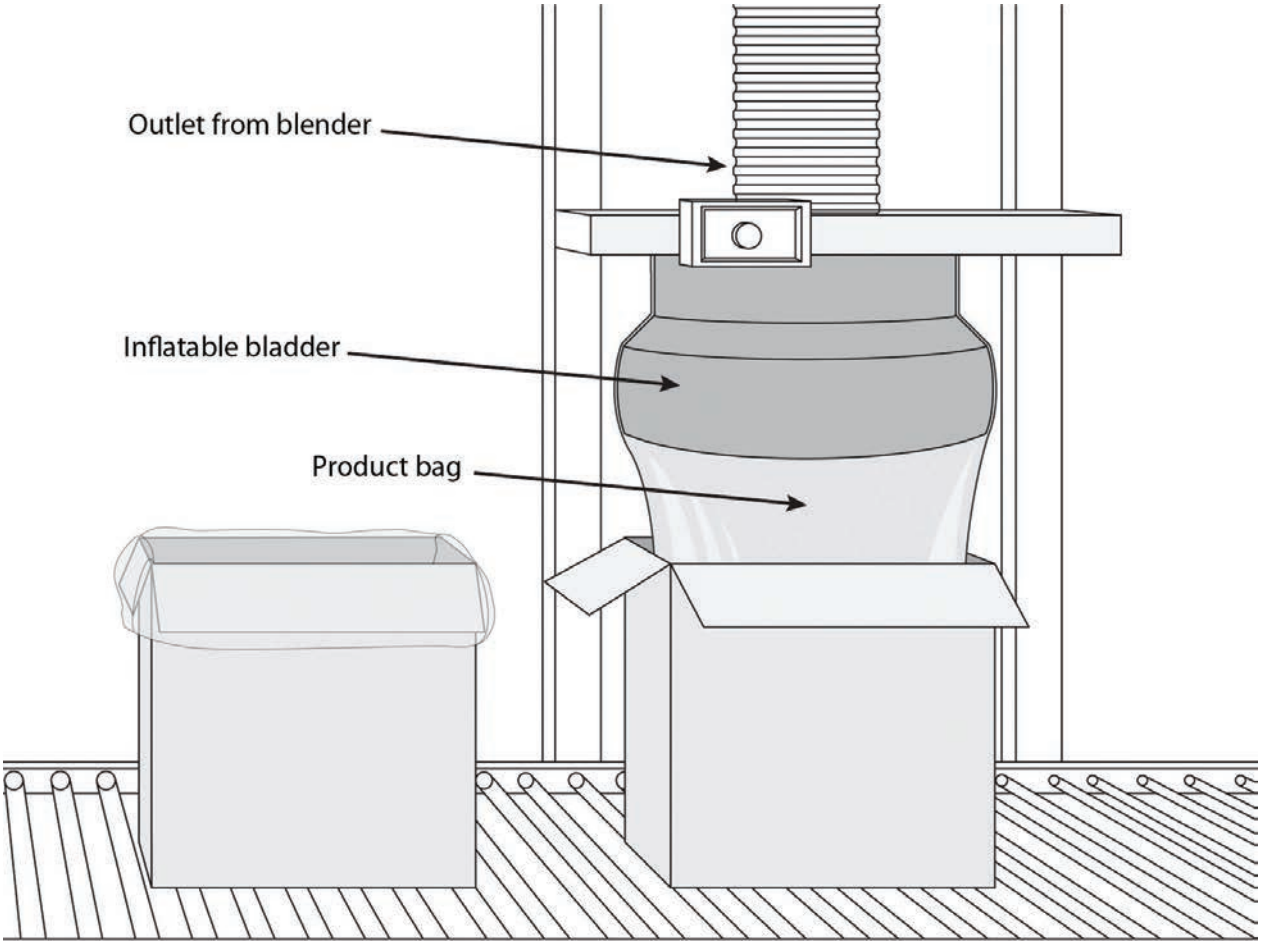


Figure 8-13. Dust control during bag filling operation

References

29 CFR 1910.133. Eye and face protection. Code of Federal Regulations. Washington, DC: U.S. Government Printing Office, Office of the Federal Register.

29 CFR 1910.134. Respiratory protection. Code of Federal Regulations. Washington, DC: U.S. Government Printing Office, Office of the Federal Register.

29 CFR 1910.134 (d)(3)(i)(A). Respiratory protection (assigned protection factors). Code of Federal Regulations. Washington, DC: U.S. Government Printing Office, Office of the Federal Register.

29 CFR 1910.138. Hand protection. Code of Federal Regulations. Washington, DC: U.S. Government Printing Office, Office of the Federal Register.

29 CFR 1910.1200. Hazard communication. Code of Federal Regulations. Washington, DC: U.S. Government Printing Office, Office of the Federal Register.

29 CFR 1910.1200 (b)(5). Hazard communication (labeling exemptions). Code of Federal Regulations. Washington, DC: U.S. Government Printing Office, Office of the Federal Register.

29 CFR 1910.1200 (h)(3). Toxic and hazardous substances (training). Code of Federal Regulations. Washington, DC: U.S. Government Printing Office, Office of the Federal Register.

29 CFR 1910.1200.A.0.4.3.1. Considerations for the classification of mixtures. Code of Federal Regulations. Washington, DC: U.S. Government Printing Office, Office of the Federal Register.

29 CFR 1910.1200.A.0.5. Bridging principles for the classification of mixtures where test data are not available for the complete mixture. Code of Federal Regulations. Washington, DC: U.S. Government Printing Office, Office of the Federal Register.

ACGIH (American Conference of Governmental Industrial Hygienists) [2007]. Industrial ventilation: a manual of recommended practice for operation and maintenance. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

ACGIH (American Conference of Governmental Industrial Hygienists) [2013]. Industrial ventilation: a manual of recommended practice. 28th ed. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Akpinar-Elci M, Travis WD, Lynch DA, Kreiss K [2004]. Bronchiolitis obliterans syndrome in popcorn production plant workers. Eur Respir J *24*(2):298–302.

Alleman T, Darcey DJ [2002]. Case report: bronchiolitis obliterans organizing pneumonia in a spice process technician. J Occup Environ Med *44*(3):215–216.

Anderson SE, Franko J, Lukomska E, Meade B [2011]. Evaluation of the hypersensitivity potential of alternative butter flavorings: are they safe alternatives for diacetyl? [abstract]. Toxicologist *120*(2):520.

Anderson SE, Franko J, Wells JR, Lukomska E, Meade BJ [2013]. Evaluation of the hypersensitivity potential of alternative butter flavorings. Food Chem Toxicol *62C*:373–381.

ANSI (American National Standards Institute) [2010]. American national standard: occupational and educational personal eye and face protection devices. New York: American National Standards Institute, Inc, ANSI Z87.1–2010.

ANSI/AIHA [2003]. American National Standard for Laboratory Ventilation. Fairfax, VA: American Industrial Hygiene Association Publication No. ANSI Z9.5–2002.

Boylstein R, Piacitelli C, Grote A, Kanwal R, Kullman G, Kreiss K [2006]. Diacetyl emissions and airborne dust from butter flavorings used in microwave popcorn production. J Occup Environ Hyg *3*(10):530–535.

Burgess WA, Ellenbecker MJ, Treitman RD [2004]. Ventilation for control of the work environment. Hoboken, NJ: Wiley-Interscience.

Cavalcanti ZD, Albuquerque Filho AP, Pereira CA, Coletta EN [2012]. Bronchiolitis associated with exposure to artificial butter flavoring in workers at a cookie factory in Brazil. Jornal Brasileiro DePneumologia *38*(3):395–399.

CDC (Centers for Disease Control and Prevention) [2002]. Fixed obstructive lung disease in workers at a microwave popcorn factory—Missouri, 2000–2002. MMWR *51*(16):345–347.

CDC (Centers for Disease Control and Prevention) [2007]. Fixed obstructive lung disease among workers in the flavor-manufacturing industry—California, 2004–2007. MMWR *56*(16):389–393.

CDC [2013]. Obliterative bronchiolitis in workers in a coffee-processing facility—Texas, 2008-2012. MMWR *62*(16):305–307.

Cecala AB, Volkwein JC, Daniel JH [1988]. Reducing bag operator's dust exposure in mineral processing plants. Appl Ind Hyg *3*(1):23–27.

Chem Service Inc. [1988]. Material safety data sheet, 2,3-pentanedione, O-818.

Dunn KH, Garcia A, Echt A [2008]. Engineering case reports: evaluation of a local exhaust ventilation system for controlling exposures during liquid flavoring production. J Occup Environ Hyg *5*(11):D103–110.

Eastern Research Group, Inc. [2008a]. Site visits related to diacetyl and flavorings that contain diacetyl: food production facility B—a commercial bakery. OSHA Docket No. 2008–0046–0069. By Eastern Research Group, Inc. Lexington, MA.

Eastern Research Group, Inc. [2008b]. Technological and economic feasibility analysis for proposed OSHA standard for diacetyl and acetoin. Draft final report. By Eastern Research Group Inc. Lexington, MA.

Elms J, Beckett P, Griffin P, Evans P, Sams C, Roff M, Curran AD [2003]. Job categories and their effect on exposure to fungal alpha-amylase and inhalable dust in the UK baking industry. AIHA J *64*(4):467–471.

EPA (Environmental Protection Agency) [2013]. Volatile organic compounds: technical overview. http://www.epa.gov/iaq/voc2.html. Date accessed: June 2013.

FEMA (The Flavor and Extract Manufacturers Association of the United States) [2012]. Respiratory health and safety in the flavor manufacturing workplace. http://www.femaflavor.org/sites/default/files/linked_files/FEMA_2012%20Respiratory%20Health%20and%20Safety.pdf. Date accessed: September 2012.

Goodfellow HD, Tähti E [2001]. Industrial ventilation design guidebook. San Diego, CA: Academic.

Halldin CN, Suarthana E, Fedan KB, Lo YC, Turabelidze G, Kreiss K [2013]. Increased respiratory disease mortality at a microwave popcorn production facility with worker risk of bronchiolitis obliterans. Plos One *8*(2).

Health and Safety Executive [2003a]. Charging reactors and mixers from a sack or keg. In: COSHH essentials: easy steps to control chemicals. London: Health and Safety Executive.

Health and Safety Executive [2003b]. Drum emptying using a drum pump. In: COSHH essentials: easy steps to control chemicals. London: Health and Safety Executive.

Health and Safety Executive [2003c]. Drum filling. In: COSHH essentials: easy steps to control chemicals. London: Health and Safety Executive.

Health and Safety Executive [2003d]. IBC filling and emptying. In: COSHH essentials: easy steps to control chemicals. London: Health and Safety Executive.

Health and Safety Executive [2003e]. Mixing liquids with other liquids or solids. In: COSHH essentials: easy steps to control chemicals. London: Health and Safety Executive.

Health and Safety Executive [2003f]. Mixing solids with other solids or liquids. In: COSHH essentials: easy steps to control chemicals. London: Health and Safety Executive.

Health and Safety Executive [2003g]. Sack emptying. In: COSHH essentials: easy steps to control chemicals. London: Health and Safety Executive.

Health and Safety Executive [2003h]. Sack filling. In: COSHH essentials: easy steps to control chemicals. London: Health and Safety Executive.

Health and Safety Executive [2003i]. Weighing and handling flour improvers, etc. (craft bakery). In: COSHH essentials: easy steps to control chemicals. London: Health and Safety Executive.

Heitbrink WA, McKinnery WN, Jr. [1986]. Dust control during bag opening, emptying and disposal. Appl Ind Hyg *1*(2):101–109.

Hirst DV, Dunn KH, Shulman SA, Hammond DR, Sestito N [2014]. Evaluation of engineering controls for the mixing of flavorings containing diacetyl and other volatile ingredients. J Occup Environ Hyg *11*(10):680–687.

Hirst N, Ryder M, Brocklebank M [2002]. Containment systems: a design guide. Rugby, Warwickshire, UK: Gulf Professional Publishing.

Hubbs AF, Cumpston AM, Goldsmith WT, Battelli LA, Kashon ML, Jackson MC, Frazer DG, Fedan JS, Goravanahally MP, Castranova V, Kreiss K, Willard PA, Friend S, Schwegler-Berry D, Fluharty KL, Sriram K [2012]. Respiratory and olfactory cytotoxicity of inhaled 2,3-pentanedione in Sprague-Dawley rats. Am J Pathol *181*(3):829–844.

Hubbs AF, Goldsmith WT, Kashon ML, Frazer D, Mercer RR, Battelli LA, Kullman GJ, Schwegler-Berry D, Friend S, Castranova V [2008]. Respiratory toxicologic pathology of inhaled diacetyl in Sprague-Dawley rats. Toxicol Pathol *36*(2):330–344.

IPCS (International Programme on Chemical Safety) [2009]. International Chemical Safety Card, 2,3-Butanedione. http://www.inchem.org/documents/icsc/icsc/eics1168.htm. Date accessed: March 2014.

Kanwal R, Kullman G, Fedan K, Kreiss K [2011]. Occupational lung disease risk and exposures to butter flavoring chemicals after implementation of controls at a microwave popcorn plant. Public Health Rep *126*:480–494.

Kanwal R, Kullman G, Piacitelli C, Boylstein R, Sahakian N, Martin S, Fedan K, Kreiss K [2006]. Evaluation of flavorings-related lung disease risk at six microwave popcorn plants. J Occup Environ Med *48*(2):149–157.

King TE, Jr. [1989]. Bronchiolitis obliterans. Lung *167*(2):69–93.

Merck Chemicals International [2010]. 2,3-Pentanedione. http://www.merck-chemicals.com/2-3-pentanedione/MDA_CHEM-818555/p_HMKb.s1LnGcAAAEWhuEfVhTl. Date accessed: December 2010.

Morgan DL, Flake GP, Kirby PJ, Palmer SM [2008]. Respiratory toxicity of diacetyl in C57BL/6 mice. Toxicol Sci *103*(1):169–180.

Morgan DL, Jokinen MP, Price HC, Gwinn WM, Palmer SM, Flake GP [2012]. Bronchial and bronchiolar fibrosis in rats exposed to 2,3-pentanedione vapors: implications for bronchiolitis obliterans in humans. Toxicol Pathol *40*(3):448–465.

National Toxicology Program [2011]. Short-term bioassay data for 2,3-butanedione (diacetyl). http://tools.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=shorttermbioassaydata.datasearch&chemical_name=2%2C3%2DButanedione%20%28Diacetyl%29&cas_no=431-03-8&study_no=C20725&study_length=90%20Days&protocol_no=2072502. Date accessed: 7/25/2011.

Naumann BD, Sargent EV, Starkman BS, Fraser WJ, Becker GT, Kirk GD [1996]. Performance-based exposure control limits for pharmaceutical active ingredients. AIHA J *57*(1):33–42.

NFPA (National Fire Protection Association) [2007]. NFPA 77: Recommended Practice on Static Electricity. Quincy, MA: National Fire Protection Association. NFPA 77.

NIOSH [2004]. NIOSH Respirator Selection Logic. By Bollinger N. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2005–100.

NIOSH [2007]. Hazard evaluation and technical assistance report: Carmi Flavor and Fragrance Company, Inc., Commerce, CA. By Kanwal R, Kullman G. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2006–0303–3043.

NIOSH [2008a]. Evaluating occupational exposures and work practices at Agilex Flavors, Inc. formerly Key Essentials, Inc., Rancho Santa Margarita, CA. A technical assistance report to the California/Occupational Safety and Health Administration. By McKernan L, Dunn K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2006–0361–2.

NIOSH [2008b]. Hazard evaluation and technical assistance report: Gold Coast Ingredients, Inc., Commerce, CA. By Bailey R, McKernan L, Dunn K, Sahakian N, Kreiss K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2007–0033–3074.

NIOSH [2008c]. In-depth survey report: evaluation of engineering controls for the mixing of flavoring chemicals: Gold Coast Ingredients, Inc., Commerce, CA. By Dunn K, Echt A, Garcia A. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for

Occupational Safety and Health, NIOSH EPHB Report No. 322–11a.

NIOSH [2008d]. In-depth survey report: evaluation of engineering controls for the production of liquid and powder flavoring chemicals. Kerry Ingredients and Flavours, Inc., formerly Mastertaste, Inc., Commerce, CA. By Dunn K, Echt A, Couch J, Myers D. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH EPHB Report No. 322–12a.

NIOSH [2009]. Hazard evaluation and technical assistance report: Chr. Hansen, Inc., New Berlin, WI. By Sahakian N, Kullman G, Dunn K, Kanwal R. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2007–0327–3083.

NIOSH [2010]. Certified Equipment List. http://www.cdc.gov/niosh/npptl/topics/respirators/cel. Date accessed: July 2010.

NIOSH [2015]. Best practices: engineering controls, work practices and exposure monitoring for occupational exposures to diacetyl and 2,3-pentanedione. By Dunn KH, McKernan LT, Garcia A. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication 2015–197.

OSHA (Occupational Safety and Health Administration) [2002]. Recommended preventative and control measures to reduce the risk of obstructive lung disease among workers in the microwave popcorn packaging industry. http://www.osha.gov/SLTC/flavoringlung/preventativecontrolmeasures_regionvii.html. Date accessed: August 2010.

OSHA [2013]. Hazard communication guidance for diacetyl and food flavorings containing diacetyl. http://www.osha.gov/dsg/guidance/diacetyl-guidance.html. Date accessed: July 2013.

Rigler MW, Longo WE [2010]. Emission of diacetyl (2,3 butanedione) from natural butter, microwave popcorn butter flavor powder, paste, and liquid products. Int J Occup Environ Health *16*(3):291–302.

Roberts DW, York M, Basketter DA [1999]. Structure-activity relationships in the murine local lymph node assay for skin sensitization: alpha,beta-diketones. Contact Dermatitis *41*(1):14–17.

SEFA [2002]. Laboratory Fume Hoods: Recommended Practices (SEFA 1-2002).

Sigma Aldrich [2010]. Material safety data sheet, No. B85037, 2,3- butanedione. http://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=D3634&brand=SIGMA&PageToGoToURL=http%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsigma%2Fd3634%3Flang%3Den. Date accessed: December 2009.

Stokes WS [2002]. Humane endpoints for laboratory animals used in regulatory testing. ILAR J *43 Suppl*(Review):S31–38.

Sugai S, Murata K, Kitagaki T, Tomita I [1990]. Studies on eye irritation caused by chemicals in rabbits—1. A quantitative structure-activity relationships approach to primary eye irritation of chemicals in rabbits. J Toxicol Sci *15*(4):245–262.

Widess E [2013]. Memorandum of January 11, 2013, from E. Widess, State of California, Department of Industrial Relations, Division of Occupational Safety and Health, to L. Taylor McKernan, Education and Information Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services

Zaccone EJ, Thompson JA, Ponnoth DS, Cumpston AM, Goldsmith WT, Jackson MC, Kashon ML, Frazer DG, Hubbs AF, Shimko MJ, Fedan JS [2013]. Popcorn flavoring effects on reactivity of rat airways in vivo and in vitro. Journal of Toxicology and Environmental Health, Part A *76*:669-689.

9 Medical Monitoring and Surveillance of Exposed Employees

Despite attempts to control exposure to diacetyl, 2,3-pentanedione, and similar flavoring compounds, some employees may develop health effects as a result of insufficient control, additive effects, intermittent peak exposures, susceptibility, unmeasured flavoring compounds in powdered form, or unrecognized hazardous exposures. Medical monitoring and surveillance of employees exposed to diacetyl and similar flavoring compounds are important, as these employees 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 for slowly progressive occupational lung diseases such as silicosis and coal worker's pneumoconiosis. The most important component of an effective medical monitoring program for an employee exposed to diacetyl and similar flavoring compounds is to carefully follow spirometry test results over time, comparing current to past test results to identify excessive declines in lung function [California Department of Public Health 2012]. Spirometry tests must be of 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 employees. The chapter also provides examples that illustrate how medical surveillance can identify workplace risk factors.

Medical Monitoring

Medical monitoring of employees, sometimes called medical screening, involves periodic medical follow up for early detection of work-related disease. The intended benefit of early detection is to identify disease in early stages when steps can still be taken to prevent progression from pre-clinical to clinical disease or from milder to more symptomatic disease. This approach is called secondary prevention because it attempts to ameliorate or at least halt the progression of health effects that have already occurred. Evidence of early disease identified through medical monitoring serves as a sentinel event or warning that other employees might be at risk for the same exposures and outcomes. This warning should stimulate efforts to evaluate the workplace to identify possible risk factors for exposures that can be controlled. Systematic evaluation and use of medical monitoring data obtained from individual employees to better protect a population of employees is an important component of the overall medical surveillance program. This approach contributes to the goal of primary prevention, to prevent disease from developing in other employees.

Medical Surveillance

The systematic analysis of aggregated results over time constitutes medical (epidemiologic) surveillance of trends in symptoms or functional changes that can be assessed in relationship to jobs, tasks, and exposures [Silverstein 1990]. For medical monitoring to serve surveillance purposes, a formal process should be in place to assure that data from a screened employee population is evaluated in aggregate at regular intervals. Epidemiologic analysis of medical

results and questionnaire and/or administrative data to evaluate for possible risk factors for disease can result in understanding what actions need to be prioritized to decrease the risk of subsets of employees and can document the effectiveness of interventions over time in preventing flavoring-related health effects.

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. This is because flavoring-related lung disease can progress rapidly and have grave consequences, so it is important to assure that the medical monitoring program director can quickly evaluate clinical data and make medical judgments about appropriate diagnostic and therapeutic measures, including medical removal. This individual (hereafter referred to as "the medical monitoring program director") should ensure that the monitoring program collects high quality data, including relevant questionnaire data and high quality spirometry tests that adhere to ATS/ERS technical guidelines for spirometry [Miller et al. 2005], or the most recent equivalent guidelines. The medical monitoring program director should also ensure that medical monitoring data is appropriately evaluated for surveillance purposes, including evaluation of aggregated results to identify risk factors and opportunities to better prevent flavoring-related lung disease.

The employer should ensure that the medical monitoring program director is familiar with the natural history of flavoring-related lung disease and is knowledgeable about operating a spirometry program that maintains high test accuracy, precision and validity. The employer should provide the following to the medical monitoring program director:

- A copy of the NIOSH Alert, "Preventing Lung Disease in Workers Who Use or Make Flavorings" [NIOSH 2003];
- A copy of this criteria document;
- A description of work areas, job categories, and work tasks;
- A description of any personal protective equipment to be used by employees; and
- Results of any environmental sampling related to potential flavorings exposures.

9.2 Employees to Include in the Medical Monitoring Program

All permanent, temporary, and contract employees who work in or enter areas where diacetyl, 2,3-pentanedione, or similar flavoring compounds or products that contain these compounds are used or produced should be included in the medical monitoring program. Employees who work in or enter these areas for a total of 40 or more hours per year should be included in the medical monitoring program. In addition to production employees, employees who are periodically exposed such as supervisors, warehouse employees, laboratory employees, quality assurance/control employees, shipping and receiving employees, maintenance employees, janitorial employees, and office employees should also be included in the program, as employees with lung function abnormalities were identified in nonproduction jobs during several NIOSH HHE investigations [Kanwal et al 2006; Kanwal et al 2011]. Employees 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 2012].

To achieve the intent of primary and secondary prevention, employers have an interest in

attaining a high rate of employee participation in regular medical monitoring. Voluntary participation should be encouraged at a time and place convenient to employees and should be provided at no cost to employees.

9.3 Medical Monitoring Program Elements

The medical monitoring evaluation should include a questionnaire to obtain health and exposure information and spirometry to assess lung function. The questionnaire data from all employees 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 such as work tasks and practices that can be addressed to decrease lung disease risk (see section 9.9).

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 program director can use information from the questionnaire when assessing the employee at each evaluation. Because employees with biopsy-documented obliterative bronchiolitis may have normal spirometry, chest symptoms such as exertional shortness of breath merit attention as suggestive of an occupational lung condition requiring employee education and follow up. Similarly, persons with abnormal spirometry, despite absent chest symptoms, may have occupational lung disease requiring attention.

Work history questions should allow employees 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 employee's exposure to diacetyl and similar flavoring compounds. For example, for an employee 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 program director develop appropriate questions on jobs and exposures, the employer should provide the medical monitoring program director with the specific job titles of potentially exposed employees, a description of the work tasks for each job that may be associated with potential for exposure to diacetyl and similar flavoring compounds, and the types of personal protective equipment (e.g., respirators) and other measures that employees have available to them to minimize exposures in each job. A visit to the plant by the medical monitoring program 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 1978] or the NHANES III questionnaire [CDC 1994] can provide standardized questions. Examples of questions NIOSH has used in HHE medical surveys of flavoring-exposed employees can be

found in NIOSH HHE reports at http://www.cdc.gov/niosh/hhe/.

While respiratory symptom information is important in the assessment of employees exposed to diacetyl and similar flavoring compounds or products that contain these compounds, the medical monitoring program director should not conclude that an employee's exposures are below harmful levels solely by the absence of respiratory symptoms. Employees may not experience respiratory symptoms early in the course of excessive lung function decline. NIOSH medical surveys of flavoring-exposed employees have identified airways obstruction [Kreiss et al. 2002] and excessive declines in lung function [NIOSH 2008] in employees who did not report respiratory symptoms. Similarly, about half of the employees with airways obstruction found in surveillance of California flavoring manufacturing employees had no chest symptoms [Kim et al. 2010]. Absence of symptoms does not negate the need for clinical differential diagnosis and evaluation of employees with spirometric abnormalities.

The medical monitoring program director should counsel employees identified as having pre-existing lung disease on their initial evaluation regarding the potential risks of working in areas where they may be exposed to diacetyl and other flavoring compounds. The medical monitoring program director should also explain that it may be hard to determine the relative contributions of work exposures vs. pre-existing lung disease to any future abnormal lung function declines. Such employees should also be referred to their personal physician for additional evaluation and recommendations regarding potential exposure to these substances.

9.3.2 Spirometry

Every employee 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 employees exposed to diacetyl and similar flavoring compounds (see section 9.6). High quality spirometry tests are necessary to allow the medical monitoring program director to correctly interpret the results and make appropriate recommendations to the employee and the employer. Accurate 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 program director.

9.3.2.1 Persons administering the spirometry examination

Each person administering spirometry examinations should successfully complete a NIOSH-Approved Spirometry Training Course (information at http://www.cdc.gov/niosh/topics/spirometry/training.html) or equivalent and maintain valid certificates. The medical monitoring program director may also benefit from this training. The ATS/ERS [Miller et al. 2005] and the American College of Occupational and Environmental Medicine (ACOEM) [Townsend 2011] endorse the content of 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. The medical monitoring program director should provide for ongoing review of test quality and feedback to technicians about opportunities for improvement. The combination of initial training, refresher training, electronic feedback from spirometers during

testing, and ongoing review of test quality with timely feedback to technicians can help a program achieve a high proportion of technically acceptable spirometry tests [Redlich et al. 2014]. Certification of acceptable spirometry test administration is an additional means of addressing quality concerns (National Board for Respiratory Care [NBRC 2016]; American Association for Respiratory Care [AARC 2011]).

9.3.2.2 Spirometer specifications

Spirometry testing equipment should meet the 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. 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/ERS protocol should be requested from the spirometer manufacturer.

9.3.2.3 Spirometry testing protocol and reporting information

Administration of spirometry tests should follow the ATS/ERS guidance for standardization of spirometry or most recent equivalent

Testing Procedures	1. Spirometer calibration checks should be performed using a currently calibrated (per manufacturer recommendations) 3-liter syringe on each day of testing [Miller et al. 2005]. A copy of the spirometer calibration report should be maintained in either electronic or hard copy form. 2. Spirometry should be performed in the same documented position (either sitting or standing) during the baseline and all subsequent tests. 3. A minimum of three forced exhalation maneuvers producing "acceptable curves" on the spirometry report should be characterized by the following: ▪ Lack of hesitation (back-extrapolation volume should be less than 5% of FVC or 150 mL, whichever is larger) ▪ No cough in the first second of the maneuver ▪ No evidence of airflow cessation, variable effort, leak, obstructed mouthpiece, positive or negative zero flow error(s), or extra breath(s) ▪ Acceptable end-of-test criteria (≤ 25 mL increase in volume for 1 second or a maneuver longer than 15 seconds) 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 [Hankinson et al. 1999] as they are based on a large sample of the U.S. population. Because 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 0.88 [Hankinson et al. 2010; Redlich et al. 2014]. In the future, it will be preferable to use Asian-specific equations for predicted values, such as from NHANES Plus data, when they are available. 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.

Figure 9-1. Spirometry guidelines for testing procedures and interpretation

[Miller et al. 2005]. These guidelines outline the criteria to follow to ensure overall test results are valid (Figure 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 should include spirometry test reports and retention of all spirometry test results in printed or electronic format. Spirometry test reports for the employee's health record should contain, at a minimum, the employee's age, height, sex, race, and weight; numerical values and volume-time and flow-volume spirograms for at least the three 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 test results and forms should also be recorded.

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 should be fully familiar with and adhere to the current ATS/ERS guidelines for instrument calibration check procedures. Calibration check procedures should include daily (day of testing) leak checks (for volume spirometers) and volume accuracy checks (performed at different speeds of injection for flow spirometers) and according to the frequency established by the current ATS/ERS spirometry standardization statement. Instrument calibration check records should be maintained by the provider for as long as the related employees' medical reports are maintained. Spirometer software should automatically perform quality assurance checks on expiratory maneuvers during each spirometry testing session. Messages should alert the technician to maneuver acceptability errors and test session nonrepeatability. Each spirometry test session should have the goal of obtaining three acceptable with two repeatable forced expiratory maneuvers, as defined by the current ATS/ERS spirometry standardization statement. Because 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 and can be accessed at http://www.cdc.gov/niosh/docs/2011-135/. Providers should utilize physicians or other qualified healthcare 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]. When suboptimal quality tests with potential for improvement are identified, the reviewing physician or other appropriate healthcare professional should provide feedback to the appropriate technician(s) along with specific 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 employees' results that are truly normal may be considered abnormal, 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 employees. Low quality spirometry has greater variability in test results; over time, decreased precision may cause the medical monitoring program director to incorrectly identify whether an employee 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 was poor and thus not useful for assessing lung function changes over time [Kanwal et al. 2011; Kreiss et al. 2012; 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 employees 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. 2012]. Employers of flavoring-exposed employees should be aware of the characteristics of high quality spirometry programs so they can evaluate the quality of spirometry services offered by medical providers, monitor performance, and take corrective actions if necessary. OSHA and NIOSH have published an information sheet on spirometry for employers [NIOSH 2011b].

9.4 Frequency of Medical Monitoring Evaluations

Newly hired employees and current employees should have baseline evaluations before they are allowed to work in or enter areas as previously described where they may be exposed to diacetyl, 2,3-pentanedione, or similar flavoring compounds. Employees in the medical monitoring program should be evaluated with a questionnaire and spirometry every 6 months due to the potentially rapid development of flavoring-related lung disease [Redlich et al. 2014]. If an employee exposed to diacetyl or similar flavoring compounds is identified as likely having lung disease from this exposure, then all employees who perform similar job tasks or have a similar or greater potential for exposure should be evaluated every 3 months. More frequent evaluation (every 3 months) is also appropriate for employees with excessive decline in FEV_1 and similarly exposed employees. Identification of flavoring-related lung disease or excessive FEV_1 decline should

also trigger an environmental assessment to identify and correct potential sources of hazardous exposures. 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 increasing monitoring physicians' 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 employees with likely flavoring-related lung disease are identified. Employees 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 that last more than 6 weeks. Such employees should be immediately evaluated by the medical monitoring program director. All employees who have been in the monitoring program should have a final evaluation at the end of employment [California Department of Public Health 2012].

9.5 Reporting Medical Monitoring Results

The medical monitoring program director or designee should review and interpret questionnaire and spirometry results, including assessing spirometry quality. During an employee's scheduled visit for a medical monitoring program evaluation, the medical monitoring program director or designee should inquire about the employee's knowledge of the potential risk from exposure to diacetyl, 2,3-pentanedione, or similar flavoring compounds and of how to minimize the risk. The medical monitoring program director or designee should educate employees as needed [California Department of Public Health 2012], and encourage employees to report any new persistent respiratory symptoms to their supervisor or the monitoring physician. At the end of each evaluation visit or as soon as possible thereafter, the medical monitoring program director should provide the employee with a written report describing the following items:

- The results of any medical tests performed on the employee
- The medical monitoring program director's opinion regarding any abnormalities detected during the evaluation and recommendations for further evaluation and treatment
- Whether or not the employee has any detected medical condition which would place the employee at increased risk to health from exposure to diacetyl, 2,3-pentanedione, or similar flavoring compounds
- Recommendations, if necessary, for reducing the employee's exposure to diacetyl, 2,3-pentanedione, or similar flavoring compounds
- Any recommended limitation upon the employee's use of personal protective equipment.

The medical monitoring program director should inform the employer in writing of the following:

- Any recommendations for limiting the employee's workplace exposures (e.g., reducing exposure to diacetyl, 2,3-pentanedione, or similar flavoring compounds by removal, or limitations of the employee's duties or activities) or on the employee's use of personal protective equipment
- A statement that the physician has informed the employee of the results of the medical examination and any medical conditions that require further evaluation or treatment.

The specific condition, issue, or concern resulting in recommendations for limiting the employee's exposure to diacetyl, 2,3-pentanedione, or similar flavoring compounds or on the employee's use of personal protective equipment should not be specified in the write-up to the employer without the employee's consent. Also, any aspect of the employee's medical history that has no bearing on whether the employee should continue to work in areas where diacetyl, 2,3-pentanedione, or similar flavoring compounds are used should not be revealed to the employer. A copy of the medical monitoring program director's written opinion provided to the employer should also be provided to the employee.

9.6 Early Identification of Affected Employees

Early recognition of employees with lung disease due to exposure to diacetyl, 2,3-pentanedione, or similar flavoring compounds is essential to prevent rapid progression to severe irreversible disease. Identifying affected employees will also stimulate prevention efforts so that risk to other employees is minimized. The most effective means for identifying affected employees early is careful evaluation of results of serial spirometry tests of employees in the medical monitoring program. Symptom reports alone are not a reliable indicator of early disease, as many employees with early disease will be asymptomatic. However, symptom reports of exertional shortness of breath can reflect pathologic obliterative bronchiolitis even when spirometry remains normal [Kreiss 2013].

At each evaluation of an employee in the medical monitoring program, the medical monitoring program 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, 2,3-pentanedione, or similar flavoring compounds is an abnormal decline in the FEV_1. An employee's longitudinal test results may reveal an abnormal decline in FEV_1 compared to baseline even when each individual test value is found to be normal because it is above the LLofN calculated from the reference population [Townsend et al 2011; Kreiss et al. 2012; Redlich et al. 2014]. While such test results might not meet the criteria for an abnormality such as airways obstruction or spirometric restriction, 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 employees with obstructive abnormalities (FEV_1/FVC ratio and FEV_1 less than the LLofN) need additional medical tests to assess whether they have obliterative bronchiolitis. Employees with restrictive abnormalities (FVC less than LLofN and normal FEV_1/FVC ratio) also need additional medical tests to differentiate between nonlung causes and lung causes of spirometric restriction, including obliterative bronchiolitis [Ghanei et al. 2008; King et al. 2011; Markopoulou et al. 2002].

The criteria for an abnormal excessive 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 [Redlich et al. 2014]. ATS/ERS and ACOEM have stated that a decline in FEV_1 over one year should exceed 15% before being considered clinically meaningful [Pellegrino et al. 2005; Townsend 2011]. 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.

Because lung disease caused by flavorings can progress rapidly, it is useful to identify those potentially at risk before so much lung function is lost [Kreiss et al. 2002; NIOSH 2006, 2007]. Some studies indicate that when ATS/ERS 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 and Petsonk [2004] found that the 5th percentile for FEV_1 declines over 6 months in all employees studied was 320 mL (7.8%). In stable employees (those employees whose FEV_1 slope over 5 years was less than 90 mL/year), it was 300 mL (7.1%). In healthy employees (those employees without symptoms or methacholine responsiveness over 5 years), it was 280 mL (6.5%). The quality of spirometry data in this study reflected a within-person variation of 3% that is rarely achievable. Within-person variation of 6% is typical for spirometry programs, and an assumption of that level of variability was used by ATS to develop its recommendation for using 15% loss of FEV_1 as a threshold [Redlich et al. 2014].

In another study that used data with a within-person variation of 4% from a spirometry surveillance program for thousands of employees at a large chemical company, Wang et al. [2006] found that the 5th 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/ERS criteria and achieves high quality spirometry, an FEV_1 decline of 10% or higher in one year or less can be considered abnormal and used as a threshold for further medical evaluation of the employee. ACOEM now accepts this 10% criterion after allowing for expected average annual loss due to aging in high risk settings when the relationship between longitudinal results and endpoint disease is clear, as in flavoring-exposed employees [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, SPIROLA, to help spirometry programs measure their within-person variation in FEV_1 as a measure of the precision of spirometry obtained by the spirometry providers (an indication of spirometry quality across the providers' programs). SPIROLA also provides a longitudinal limit of decline (LLD) for each individual tested, a threshold for determining abnormal loss of FEV_1 that is adjusted for the quality of the provider's spirometry program [NIOSH 2010]. The LLD allows the spirometry provider to determine if an individual's serial spirometry results suggest an excessive decline in lung function and allows higher quality programs to identify smaller changes in lung function as abnormal (http://www.cdc.gov/niosh/topics/spirometry/spirola-software.html). The advantage of using relative lower LLD and 5th percentile approaches over the 15% criterion in flavorings-exposed microwave popcorn employees has been demonstrated [Chaisson et al. 2010].

9.7 Continuity of Medical Monitoring

Employers may change medical providers of medical monitoring services. Employers should ensure that prior medical monitoring program directors transfer medical monitoring records, including spirometry tests and questionnaires, to new medical monitoring program directors. If necessary to gain access, employers or new providers should ask employees to sign releases allowing new providers to obtain previous medical monitoring and surveillance records from previous provider(s).

9.8 Tests Used in Medical Monitoring

9.8.1 Spirometry

The first step in evaluating an employee whose medical monitoring spirometry test shows either an excessive decline in FEV_1 (even if individual test results are still above the LLofN) or a new abnormality (e.g., obstructive, restrictive, or mixed spirometric abnormality) compared to baseline is to repeat the test within one month to confirm the change. If the repeat spirometry test confirms an excessive decline in FEV_1 or other abnormality, the employee should be referred for more extensive pulmonary function tests (PFTs) (described below). The medical monitoring program director may request these and other necessary tests or refer the employee to a pulmonary medicine physician at no cost to the employee.

9.8.2 Other Pulmonary Function Tests

The referred employee should receive complete PFTs that include spirometry with an assessment of bronchodilator response, DLCO, and static lung volumes. Most employees who have developed lung disease while being exposed to diacetyl and similar flavoring compounds have not had a response to bronchodilator [Akpinar-Elci et al. 2004; Kim et al. 2010]. In other words, they had fixed airways obstruction with an FEV_1 and/or FVC increase less than 12% and 200 mL after bronchodilator) [Pellegrino et al. 2005]. DL_{CO} in affected employees with airways obstruction has usually been normal, although some individuals with advanced disease have had a low DL_{CO} [Akpinar-Elci et al. 2004]. 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.

9.8.3 High-resolution Computerized Tomography

Employees 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 employees with fixed airways obstruction. Other common findings include cylindrical bronchiectasis, bronchial wall thickening, and a mosaic pattern of attenuation. Centrilobular nodules may also be seen [Cox et al. 2014]. Patchy ground glass opacities have been observed less commonly. These findings may not be present despite obliterative bronchiolitis documented by biopsy [King et al. 2011]. HRCTs have not been systematically performed in flavoring-exposed employees with restrictive pulmonary function abnormalities or with excessive FEV_1 declines within the normal range of FEV_1. Specialist consideration of the diagnostic utility of this test is suggested.

9.8.4 Lung Biopsy

It is not routinely necessary to obtain a lung biopsy to diagnose obliterative bronchiolitis in employees exposed to diacetyl or 2,3-pentanedione when spirometry and HRCT results are consistent with the diagnosis. While some physicians might desire biopsy confirmation, it is important to recognize that the patchy nature of obliterative bronchiolitis and lack of familiarity of some pathologists with the techniques necessary to identify bronchiolar lesions may prevent identification of the disease on biopsy. HRCT has become the method of choice for assessing

bronchiolar morphology, often replacing surgical lung biopsy [King and Kinder 2008].

Physicians caring for another population at high risk for obliterative bronchiolitis, lung transplant patients, use a similar noninvasive approach. Obliterative bronchiolitis 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]. The term bronchiolitis obliterans syndrome has also been applied to flavoring-exposed employees without surgical lung biopsies [Akpinar-Elci et al. 2004; van Rooy et al. 2007], but may lead to confusion because flavoring-related obliterative bronchiolitis differs in natural history from post-transplant bronchiolitis obliterans syndrome, which is relentlessly progressive.

There are some situations, described in the next section, where lung biopsy is appropriate for diagnosis. To obtain adequate tissue for diagnosis, a thoracoscopic or open lung biopsy should be obtained. Obtaining wedge biopsies from multiple lobes is recommended, as this approach increases the diagnostic yield [Devakonda et al. 2010]. Transbronchial lung biopsies are not useful for evaluating clinical obliterative bronchiolitis in employees exposed to diacetyl, 2,3-pentanedione, or similar compounds.

9.8.5 Determining Diagnosis Responsible for Lung Disease

Determination of the diagnosis responsible for lung disease in an employee exposed to diacetyl, 2,3-pentanedione, or similar flavoring compounds 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 employee's illness over time, and medical, work, and personal risk factor history.

In an exposed employee with evidence of clinical obliterative bronchiolitis on PFTs or HRCT scans and no other identifiable cause for the disease, biopsy is not necessary. The noninvasive clinical findings alone are sufficient to conclude that an exposed employee likely has clinical obliterative bronchiolitis and should no longer be exposed to diacetyl, 2,3-pentanedione, or similar flavoring compounds. When clinically apparent lung disease occurs in several employees at a particular plant, the need for biopsy confirmation in each employee is usually unnecessary.

When HRCT is normal in dyspneic employees, particularly if the PFTs are restrictive or normal, lung biopsy has a role. Some medical surveys of flavoring-exposed employees have revealed an increased prevalence of an isolated restrictive pattern on spirometry (i.e., without concurrent airways obstruction), but static lung volume measurements of TLC and biopsies have not been available in these studies to confirm restrictive lung disease [Kreiss 2012; NIOSH 2009, 2011c]. The evidence for restrictive and normal pulmonary functions in obliterative bronchiolitis is in patients exposed to other lung hazards, such as sulfur mustard gas and in U.S. soldiers serving in Iraq and Afghanistan, some of whom had sulfur dioxide exposure. Despite evidence from three biopsy-confirmed case series of obliterative bronchiolitis [Ghanei et al. 2008; King et al. 2011; Markopoulou et al. 2002], many pulmonary and occupational medicine specialists are not aware of the range of spirometric findings in this disease and may be reluctant to diagnose obliterative bronchiolitis in patients with

spirometric restriction or normal spirometry without pathologic confirmation. Employees who develop restrictive abnormalities or who have excessive parallel FEV_1 and FVC declines should have assessment of lung volumes, diffusing capacity, and HRCT to differentiate between restrictive lung disease and other causes of restrictive spirometric patterns. Further evaluation of restrictive lung disease for a specific diagnosis should be pursued as clinically appropriate and may require biopsy. Case reports of pathologic findings in dyspneic flavoring-exposed employees with restrictive or normal spirometry will be of interest in further guidance for clinicians responsible for the lung health of such employees.

The evaluating physician should exclude alternative causes of respiratory disease such as work-related asthma (new onset asthma or exacerbation of pre-existing asthma). An employee 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 workplace exposures. If an employee with asthma symptoms does not have changes over time on medical monitoring spirometry, a methacholine or mannitol challenge test may be necessary to determine if the employee has airways hyperresponsiveness as occurs in asthma. Worsening symptoms in an employee with pre-existing asthma may be due to exposure to diacetyl, similar flavoring compounds, or other agents in the workplace [Sahakian et al. 2008]. An important consideration for diacetyl-exposed employees 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 obliterative bronchiolitis. An employee 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 2007]. Employees 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 obliterative bronchiolitis are present. However, because HRCT abnormalities may be insensitive in detecting early or mild disease, such asthmatic employees require careful and frequent follow-up [King et al. 2011].

An employee exposed to diacetyl, 2,3-pentanedione, or similar flavoring compounds who has normal pre-exposure spirometry and subsequently develops fixed airways obstruction and has evidence of air trapping on complete PFTs or on HRCT scan, or has an excessive decline in FEV_1 and whose pulmonary function does not improve after exposure cessation, likely has clinical obliterative bronchiolitis due to this exposure.

In exposed employees who smoke, fixed airways obstruction should not be attributed to smoking if there is no evidence of emphysema on medical tests. 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 employees, work history, clinical course, and medical tests are important in attempting to differentiate between smoking-related COPD and flavoring-related obstruction. Smoking explains about 80% of COPD in the United States, with about 15% attributable to work exposures. Smoking diacetyl-exposed employees appear to have lower excess risk of obstruction than never-smoking flavoring-exposed employees [Kreiss et al. 2002].

9.9 Response to Identification of Work-related Lung Disease

Employees with abnormalities identified on medical monitoring spirometry should be counseled about the risks of further exposure and that removal from exposure is prudent because of the irreversibility of the disease, short latency, and often rapid progression. Employees who receive a diagnosis of flavoring-related lung disease or who have findings on medical evaluation that indicate likely clinical obliterative bronchiolitis or other lung disease due to workplace exposures should be placed on work restrictions to prevent any further exposure to flavoring compounds or other substances in the workplace that may cause their lung disease to worsen. Personal protective equipment is the least effective means for controlling employee exposures. The proper use of personal protective equipment requires a high level of employer and employee involvement and commitment to be effective. The use of respiratory protection is not equivalent to removal from exposures because employees may still be exposed due to incomplete compliance, selection of an inappropriate respirator, or respirator malfunction [California Department of Public Health 2012]. If possible, employers should offer affected employees the opportunity to transfer to available jobs in work areas that have minimal or nonexistent exposures. Such employees should retain seniority, wages, and benefits.

Employers of an employee with confirmed or likely flavorings-related lung disease should arrange for an industrial hygiene evaluation of the plant areas where the employee 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 employees from developing work-related lung disease. Medical monitoring evaluations of employees 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 employees with likely flavoring-related lung disease are identified (see section 9.4).

When informed, employers should record all flavoring-related lung disease cases in the OSHA Form 300 Logs of Work-Related Injuries and Illnesses.

9.10 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 employees 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 a medical monitoring and surveillance program, which includes the use of epidemiologic techniques for analyses of aggregated data obtained from evaluations of all employees 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 employees develop significant lung disease. Because production processes and work practices in manufacturing plants that use diacetyl or similar flavoring compounds or products that contain these compounds vary from plant to plant, medical surveillance may also allow identification of risk factors unique to

a particular plant. For these reasons, systematic evaluation of medical monitoring data is an important component of medical monitoring and surveillance programs for employees exposed to diacetyl or similar flavoring compounds. If the medical monitoring program director is not able to conduct such analyses, the employer or medical monitoring program 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 employees were first identified as being at risk for severe fixed airways obstruction consistent with clinical obliterative bronchiolitis from exposure to butter flavoring vapors (index facility G), four known affected former employees had worked in the mixing room as mixers of oil and butter flavorings, and four other affected former employees had worked on the packaging lines near the mixing room. A medical survey of current employees 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 employees. The environmental assessment showed that air concentrations of the butter flavoring compound 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 employees, the highest prevalence of airways obstruction was found in QC laboratory employees, five of six (83%) of whom had airways obstruction [Kreiss et al. 2002]. These employees 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 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 employees 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 2003]. 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 employees' exposures may be substantially different from those of other production employees; diacetyl air concentrations alone may not be a satisfactory predictor of risk for these employees. Because of this evidence of risk to QC laboratory employees, 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 employees in one plant [Kanwal et al. 2006]. No other plant had an elevated prevalence of airways obstruction in QC employees. Fewer bags of microwave popcorn were popped per employee per day in those plants, and the mean time-weighted average diacetyl air concentrations in the QC laboratories were lower than at index facility G.

Example 2. At a microwave popcorn plant where a young mixing room employee developed moderately severe fixed airways obstruction and other findings consistent with clinical obliterative bronchiolitis, management had put a mandatory respirator use policy for mixing room employees 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 employees 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 employees reported ever having worked as a mixer; six had abnormal lung function on NIOSH spirometry tests. The reported percentages of time these employees used respirators during these activities ranged from 0% to 100%. The median reported percentage of time was 20% for all activities, except for those where other ingredients (not flavorings) were poured into tanks in the mixing room where the median was 50% [NIOSH 2004a]. These results showed that employees were not fully compliant with management's respirator use policy; management was able to address this problem through employee 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 employees' exposures to butter flavoring compounds. (Before 2001 when NIOSH informed microwave popcorn companies of the risk of severe lung disease to employees 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 employees many years earlier to prevent severe eye irritation that employees had experienced when handling certain flavorings.)

Thus, analysis of population data generated by medical monitoring and surveillance programs plays an important role in primary prevention by helping employers of flavoring-exposed employees to recognize and take steps to characterize and correct hazardous conditions. Recognition can require epidemiologic evaluation of medical monitoring, population, and environmental data. It is therefore important for employers to ensure that this applied epidemiology is provided as part of the medical monitoring and surveillance program.

References

AARC (American Association for Respiratory Care) [2011]. Office spirometry certificate application. https://secure.aarc.org/osc/training.cfm. Date accessed: July 2016.

Akpinar-Elci M, Travis WD, Lynch DA, Kreiss K [2004]. Bronchiolitis obliterans syndrome in popcorn production plant workers. Eur Respir J *24*(2):298–302.

Banks DE, Wang ML, McCabe L, Billie M, Hankinson J [1996]. Improvement in lung function measurements using a flow spirometer that emphasizes computer assessment of test quality. J Occup Environ Med *38*(3):279–283.

California Department of Public Health [2012]. Medical surveillance for flavorings-related lung disease among flavor manufacturing workers in California. http://www.cdph.ca.gov/programs/ohb/Documents/flavorguidelines.pdf. Date accessed: July 2016.

CDC (Centers for Disease Control and Prevention) [1994]. Plan and operation of the third national health and nutrition examination survey, 1988–1994. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics. http://www.cdc.gov/nchs/nhanes/nh3rrm.htm. Date accessed: July 2016.

Chaisson NF, Kreiss K, Hnizdo E, Hakobyan A, Enright PL [2010]. Evaluation of methods to determine excessive decline of forced expiratory volume in one second in workers exposed to diacetyl-containing flavorings. J Occup Environ Med *52*(11):1119–1123.

Cox CW, Rose CS, Lynch DA [2014]. State of the art: Imaging of occupational lung disease. Radiology *270*(3):681–696.

Devakonda A, Raoof S, Sung A, TravisWD, Naidich D [2010]. Bronchiolar disorders: a clinical-radiological diagnostic algorithm. Chest *137*(4):938–951.

Enright PL, Johnson LR, Connett JE, Voelker H, Buist AS [1991]. Spirometry in the Lung Health Study. 1. Methods and Quality-Control. Am Rev Resp Dis *143*(6):1215–1223.

Estenne M, Maurer JR, Boehler A, Egan JJ, Frost A, Hertz M, Mallory GB, Snell GI, Yousem S [2002]. Bronchiolitis obliterans syndrome 2001: An update of the diagnostic criteria. J Heart Lung Transplant *21*(3):297–310.

Ferris BG [1978]. Epidemiology standardization project (American Thoracic Society). Am Rev Respir Dis *118*(6 Pt 2):1–120.

Ghanei M, Tazelaar HD, Chilosi M, Harandi AA, Peyman M, Akbari HM, Shamsaei H, Bahadori M, Aslani J, Mohammadi A [2008]. An international collaborative pathologic study of surgical lung biopsies from mustard gas-exposed patients. Respir Med *102*(6):825–830.

Hankinson JL, Kawut SM, Shahar E, Smith LJ, Stukovsky KH, Barr RG [2010]. Performance of American Thoracic Society-recommended spirometry reference values in a multiethnic sample of adults: the multi-ethnic study of atherosclerosis (MESA) lung study. Chest *137*(1):138–145.

Hankinson JL, Odencrantz JR, Fedan KB [1999]. Spirometric reference values from a sample of the general U.S. population. Am J Respir Crit Care Med *159*(1):179–187.

Kanwal R, Kullman G, Fedan K, Kreiss K [2011]. Occupational lung disease risk and exposures to butter flavoring chemicals after implementation of controls at a microwave popcorn plant. Public Health Rep *126*:480–494.

Kanwal R, Kullman G, Piacitelli C, Boylstein R, Sahakian N, Martin S, Fedan K, Kreiss K [2006]. Evaluation of flavorings-related lung disease risk at six microwave popcorn plants. J Occup Environ Med *48*(2):149–157.

Kim T, Materna B, Prudhomme J, Fedan K, Enright PL, Sahakian N, Windham G, Kreiss K [2010]. Industry-wide medical surveillance of California flavoring manufacturing workers: cross-sectional results. Am J Ind Med *53*(9):857–865.

King MS, Eisenberg R, Newman JH, Tolle JJ, Harrell FE, Jr., Nian H, Ninan M, Lambright ES, Sheller JR, Johnson JE, Miller RF [2011]. Constrictive bronchiolitis

in soldiers returning from Iraq and Afghanistan. N Engl J Med *365*(3):222–230.

King T, Kinder BW [2008]. Bronchiolitis. In: Fishman AP, ed. Fishman's pulmonary diseases and disorders. 4th ed. New York: McGraw-Hill, pp. 887–911.

Kreiss K [2012]. Respiratory disease among flavoring-exposed workers in food and flavoring manufacture. Clin Pulm Med *19*(4):165–173.

Kreiss K [2013]. Occupational causes of constrictive bronchiolitis. Curr Opin Allergy Clin Immunol *13*(2):167–172.

Kreiss K, Fedan KB, Nasrullah M, Kim TJ, Materna BL, Prudhomme JC, Enright PL [2012]. Longitudinal lung function declines among California flavoring manufacturing workers. Am J Ind Med *55*(8):657–668.

Kreiss K, Gomaa A, Kullman G, Fedan K, Simoes EJ, Enright PL [2002]. Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. N Engl J Med *347*(5):330–338.

Malmstrom K, Peszek I, Botto A, Lu S, Enright PL, Reiss TF [2002]. Quality assurance of asthma clinical trials. Control Clin Trials *23*(2):143–156.

Markopoulou KD, Cool CD, Elliot TL, Lync DA, Newell JD, Jr., Hale VA, Brown KK, Schwarz MI, Tuder RM [2002]. Obliterative bronchiolitis: varying presentations and clinicopathological correlation. Eur Respir J *19*(1):20–30.

Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J [2005]. ATS/ERS Task Force. Standardisation of spirometry. Eur Respir J *26*(2):319–338.

NBRC (National Board for Respiratory Care) [2016]. Certification examination for pulmonary function technologists. http://www.nbrc.org/rpft/pages/default.aspx. Date accessed: July 2016.

NIOSH [2003]. NIOSH alert: preventing lung disease in workers who use or make flavorings. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2004–110.

NIOSH [2004a]. Hazard evaluation and technical assistance report: American Pop Corn Company, Sioux City, IA. By Kanwal R, Boylstein R, Piacitelli C. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2001–0474–2943.

NIOSH [2004b]. Hazard evaluation and technical assistance report: ConAgra Snack Foods, Marion, OH. By Kanwal R, Kullman G. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2003–0112–2949.

NIOSH [2006]. Hazard evaluation and technical assistance report: Gilster-Mary Lee Corporation, Jasper, MO. By Kanwal R, Kullman G, Fedan K, Kreiss K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2000–0401–2991.

NIOSH [2007]. Hazard evaluation and technical assistance report: Carmi Flavor and Fragrance Company, Inc., Commerce, CA. By Kanwal R, Kullman G. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2006–0303–3043.

NIOSH [2008]. Hazard evaluation and technical assistance report: Gold Coast Ingredients, Inc., Commerce, CA. By Bailey R, McKernan L, Dunn K, Sahakian N, Kreiss K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2007–0033–3074.

NIOSH [2009]. Hazard evaluation and technical assistance report. Report on an investigation of buttermilk flavoring exposures and respiratory health at a bakery mix production facility, General Mills, Los Angeles, CA. By Day G, Cummings K, Kullman G. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2008–0230–3096.

NIOSH [2010]. Spirometry in the occupational setting: Spirometry Longitudinal Data Analysis (SPIROLA) software. Atlanta, GA: Centers for Disease Control and Prevention, National Institute for Occupational Safety

and Health, Version 3.0, updated November 2010. http://www.cdc.gov/niosh/topics/spirometry/spirola.html. Date accessed: July 2016.

NIOSH [2011a]. Get valid spirometry results EVERY time. By Beeckman-Wagner L, Freeland D, Sha Das M, Thomas K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2011–135.

NIOSH [2011b]. OSHA-NIOSH Info Sheet: Maximize your spirometry screening and surveillance resources. Morgantown, WV: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, DHHS (NIOSH) Publication No. 2011-133. http://www.cdc.gov/niosh/docs/2011-133/. Date accessed: July 2016.

NIOSH [2011c]. Hazard evaluation and technical assistance report: lung function (spirometry) testing in employees at a flavorings manufacturing plant—Indiana. By Kreiss K, Piacitelli C, Cox-Ganser J. Morgantown, WV: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2008–0155–3131.

Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF, Wanger J [2005]. Interpretative strategies for lung function tests. Eur Respir J *26*(5):948–968.

Redlich CA, Tarlo SM, Hankinson JL, Townsend MC, Eschenbacher WL, Von Essen SG, Sigsgaard T, Weissman DN [2014]. Official American Thoracic Society technical standards: spirometry in the occupational setting. Am J Respir Crit Care Med *189*(8):983–993.

Sahakian N, Kullman G, Lynch D, Kreiss K [2008]. Asthma arising in flavoring-exposed food production workers. Int J Occup Med Environ Health *21*(2):173–177.

Silverstein MA [1990]. Medical screening, surveillance, and the prevention of occupational disease. J Occup Med *32*(10):1032–1036.

Townsend MC, Occupational and Environmental Lung Disorders Committee [2011]. Spirometry in the occupational health setting—2011 update. J Occup Environ Med *53*(5):569–584.

van Rooy FG, Rooyackers JM, Prokop M, Houba R, Heederik DJ [2007]. Bronchiolitis obliterans syndrome in chemical workers producing diacetyl for food flavorings. Am J Respir Crit Care Med *176*(5):498–504.

Wang ML, Avashia BH, Petsonk EL [2006]. Interpreting periodic lung function tests in individuals: the relationship between 1- to 5-year and long-term FEV_1 changes. Chest *130*(2):493–499.

Wang ML, Petsonk EL [2004]. Repeated measures of FEV_1 over six to twelve months: what change is abnormal? J Occup Environ Med *46*(6):591–595.

Wise RA [2008]. Chronic obstructive pulmonary disease: clinical course and management. In: Fishman AP, ed. Fishman's pulmonary diseases and disorders, 4th ed. New York: McGraw-Hill, pp. 729–746.

This page intentionally left blank.

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, employee involvement, and occupational safety and health expertise. A safety and health program designed to protect employees from the adverse effects of exposure to diacetyl, 2,3-pentanedione, and other flavoring compounds 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 program, so is exposure monitoring. Exposure monitoring should be conducted whenever there is workplace exposure to diacetyl or 2,3-pentanedione.

10.1 Exposure Monitoring Program Goals

A workplace exposure monitoring program should have clear, stated goals [Mulhausen and Damiano 1998]. Site-specific exposure assessment strategies should be developed to accomplish each of these goals: (1) to determine employee exposure to diacetyl, 2,3-pentanedione, and other flavoring compounds used in the workplace; (2) to evaluate the effectiveness of work practices and engineering controls; and (3) to facilitate selection of appropriate personal protective equipment, if needed. Each of these goals requires a different sample strategy with different parameters (see Section 10.2). 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 compounds. 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 employee 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. The sampling strategy should be developed to facilitate data analysis and interpretation for the specific exposure assessment goal.

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 a comprehensive assessment to identify and quantify exposure hazards throughout a designated plant or work area to protect employees' 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 compounds 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 employees who report symptoms or illnesses

Sampling can also be used to assess any fugitive emissions from plant processes into the surrounding community.

Exposure monitoring should be conducted by qualified professionals. The sampling strategy should provide an opportunity to determine each employee's exposure, either by direct measure using personal breathing zone samples or through reasonable estimates based on the sampling of similar work tasks or jobs. Sampling strategies that group employees 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 employees 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 employee exposures are below an OEL, a compliance sampling strategy, and/or a "focused strategy," that targets employees 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 OEL if maximum-risk employees 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 compounds 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 compounds present in workplace air can be obtained from reviews of product ingredient lists, flavor or food recipes, SDSs, 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 SDSs for all flavorings and other ingredients used, although some flavoring SDSs 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 employees 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 sample and measure should be based upon the chemical, physical, and toxicological properties as well as the chemical quantities in use. For example, industry reference materials may provide helpful information on which flavoring compounds to use or avoid [FEMA 2012]. Other databases that might prove helpful may include but are not limited to National Library of Medicine (Hazardous Substances Data Bank and ChemIDplus Lite, Agency for Toxic Substances and Disease Registry (Toxicological Profiles), U.S. Environmental Protection Agency (Superfund Chemical Data Matrix). Diacetyl, 2,3-pentanedione, and other flavoring compounds can be present in air as solids, liquids, gases/vapors, or a combination of these. The physical state of the flavoring compound 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 employee exposures are below existing OELs, a focused or compliance sampling strategy that targets employees perceived to have the highest exposures may be more efficient than other strategies if maximum-risk employees and time periods can be accurately identified. Focused sampling, including personal breathing zone sampling, may also help identify short-duration tasks involving high flavoring compound 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 compound exposure source; (2) employee 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 employees according to similar exposure potential, uniform job titles, or functional job categories, the industrial hygienist should select at random a predetermined number of employees from each group for personal air sampling

to represent the exposures of those groups [Mulhausen and Damiano 1998; NIOSH 1977].

Area sampling may be useful for determining sources of airborne contaminants and identifying the worst-case chemical concentrations in various locations or processes. Selection of which employees or work locations should be sampled can help to characterize (confirm or refute) suspected areas of potential concern.

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 continues to evolve, 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 published by NIOSH at http://www.cdc.gov/niosh/nmam/ and by OSHA at http://www.osha.gov/dts/sltc/methods/index.html are described in detail in Chapter 2 of this document and are presented in Appendices A–E. 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 I.

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; 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.

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, and 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 consistently 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 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, care should be taken to avoid contaminating field samples. 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 compounds [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 that 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, 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 full work-shift sample. Work shifts that exceed 8 hours require extended sampling duration.

When the potential for exposure to diacetyl, 2,3-pentanedione, or flavoring compounds is sporadic throughout a work shift, short-term or task-based sampling may be needed to replace or supplement full-shift sampling. Short-term samples for diacetyl and 2,3-pentanedione can be collected for 15 minutes in duration. Data from these short-term measurements and other task-based sampling 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. 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 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 compound analytes such as diacetyl are light sensitive and should be protected from light during sample collection and 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 substances. 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 employees' exposures relative to an OEL. As discussed in the section of this document describing the development of the RELs, an 8-hour TWA measurement in excess of 5 ppb diacetyl or 9.3 ppb 2,3-pentanedione indicates that the employee in question was at a greater risk of developing occupationally induced illness. A 15-minute 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. Employers should monitor employees in such a fashion that he has a high degree of confidence that a very high percentage of actual daily exposures are below the REL. In statistical terms, the employer should try to attain 95% confidence that no more than 5% of employee days are over the REL.

10.3.2 Notification of Employees

Employers should establish procedures for the timely notification of employees of their environmental monitoring results or results that represent their work group, any identified exposure hazards, and any subsequent actions taken based on this monitoring to reduce their exposures. Employees should be informed about any products or processes that may generate high concentrations of diacetyl, 2,3-pentanedione, or other flavoring compounds and any PPE and changes in work practices needed in response. Employers should ensure that employees 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 employees receive and comprehend this information.

References

ACGIH (American Conference of Governmental Industrial Hygienists) [2001]. Air-sampling instruments for evaluation of atmospheric contaminants, 9th ed. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

FEMA [2012]. Respiratory health and safety in the flavor manufacturing workplace. In: The flavor and extract manufacturers association of the United States. Washington, DC: p. 28

Gross ER, Pechter E [2002]. Evaluation. In: Plog BA, ed. Fundamentals of industrial hygiene. 5th ed. National Safety Council, pp. 487–519.

Hewett P, Logan P, Mulhausen J, Ramachandran G, Banerjee S [2006]. Rating exposure control using Bayesian decision analysis. J Occup Environ Hyg *3*(10):568–581.

Jennison EA, Kullman GJ, Parker JE [1996]. Site visits. In: Harber P, Schenker M, Balmes J, eds. Occupational and environmental respiratory disease. New York: Mosby, pp. 140–147.

Milz SA, Conrad RG, Soule RD [2003]. Principles of evaluating worker exposure. In: DiNardi SR, ed. The occupational environment: its evaluation, control, and management. Fairfax, VA: The American Industrial Hygiene Association, pp. 116–128.

Mulhausen JR, Damiano J [1998]. A strategy for assessing and managing occupational exposures, 2nd ed. Fairfax, VA: American Industrial Hygiene Association, pp. 15–56.

NIOSH [1977]. Occupational exposure sampling strategy manual. Cincinnati, OH: U.S. Department of Health, Education, and Welfare, Center for Disease Control, National Institute for Occupational Safety and Health, DHEW (NIOSH) Publication No. 77–173.

NIOSH [1994]. NIOSH manual of analytical methods (NMAM). 4th ed. Schlecht PC, O'Connor PF, eds. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication 94–113 (August, 1994); 1st Supplement Publication 96–135; 2nd Supplement Publication 98–119; 3rd Supplement 2003–154.

Snedecor GW, Cochran WG [1967]. Statistical methods. Ames, IA: The Iowa State University Press, pp. 111–119.

Soule RD [2000]. Workplace sampling and analysis. In: Patty's industrial hygiene. 5th ed. New York: John Wiley and Sons, pp. 265–315.

11 Research Needs

In this chapter, knowledge gaps pertaining to diacetyl, 2,3-pentanedione and flavoring-induced lung disease are identified. General areas of need include environmental research to better measure and control exposures to flavoring substances, clinical and field studies on the epidemiology of flavoring-induced diseases, research related to personal protective equipment, and toxicological studies concerning the etiology of flavoring-related diseases.

Research is needed to characterize 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. This research should address questions such as:

- Can a more sensitive analytical 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 compounds?
- How does one effectively measure exposure to airborne particulate for diacetyl and other flavoring compounds? Would sampling and analytical methods be influenced by particle size?
- Can a real-time, portable sampling device be developed that 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?
- What are appropriate variability estimates for occupational exposures in food production facilities?
- What jobs have peak flavoring exposures that may be pertinent to health risks?
- What are the major food production processes involving flavorings that require engineering controls?
- What are the exposures for the downstream employees in food production processes or workplaces?
- What work practice interventions most effectively reduce employee exposure?

Clinical and field research studies should address such questions as:

- Is the asthma excess in flavoring employees a misdiagnosis of fixed obstruction or part of the range of flavoring-related diseases or their natural history?
- What flavoring or other chemicals are responsible for the increased prevalence in restrictive spirometry seen in one flavoring manufacturing employee population?
- To what extent does the spectrum of diacetyl-related lung disease include restrictive lung disease?
- Because obliterative bronchiolitis can be present pathologically with normal spirometric measures, should exposed employees with exertional shortness of breath be removed from further flavoring exposure or followed more intensively by clinicians until the natural history becomes clear?

- What is the natural history of flavoring-induced illness with continuing exposures, and with cessation of exposure?
- Do biomarkers of flavoring exposure or lung injury exist that could be used in employee screening or diagnosis?
- Are there genetic or epigenetic markers for susceptibility for diacetyl-related respiratory effects?
- Can longitudinal examination of spirometry in flavoring-exposed employees for excessive declines be effective in primary and secondary prevention of lung impairment in flavoring employees? What minimum quality requirements in spirometry equipment, technician performance, interpretation, and physician follow-up are necessary for flavoring-exposed employee medical surveillance to be effective?
- Can the effectiveness of a proposed standard, given the limitations of risk assessment, be substantiated by employee medical surveillance?
- Should flavoring-exposed employees undertake their personal medical surveillance with peak flow meters or portable spirometers?
- Could mortality studies of flavoring employees elucidate other potential flavoring-related risks such as kidney toxicity or burden of respiratory mortality?
- What is the prevalence of increased respiratory morbidity in employees making scented candles, hard candies, snack foods, dairy products, baked goods, e-cigarettes, fragrances, etc.?
- What nonflavoring, volatile chemicals have similar inhalation toxicity for employees in industries already shown to have excess obstructive lung disease in population-based studies such as NHANES or in clusters of obliterative bronchiolitis in specific industries, such as plastic-reinforced glass fibers in boat building or in U.S. soldiers returning from Iraq and Afghanistan?

Research needs have been identified in the area of respirators and other personal protective equipment that will continue to have an important role in employee protection.

- What methodology should be used for respirator selection for mixed chemical environments?
- 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 in mixed chemical environments?
- What are the end-of-service indicators for respirators used in mixed environments?

Unanswered questions about the mechanisms of health effects of diacetyl and 2,3-pentanedione, include the following:

- What are the chronic respiratory toxicological and pathophysiological effects of diacetyl inhalation?
- Can a CFD-PBPK model be developed for diacetyl or 2,3-pentanedione absorption during chronic exposure?
- What are the roles of metabolism and adhesion molecules in diacetyl or 2,3-pentanedione toxicity?
- Do biomarkers of flavoring exposure or lung injury exist that could be used in employee screening or diagnosis?
- Are there genetic or epigenetic markers for susceptibility for diacetyl-related respiratory effects?
- What is the role of protein damage in diacetyl or 2,3-pentanedione toxicity?

- What is the role of immunotoxicity in diacetyl or 2,3-pentanedione toxicity?
- What is the relationship between the chemical structure of diacetyl and 2,3-pentanedione and pulmonary toxicity?
- Do nonflavoring volatile workplace chemicals implicated in causing obstructive lung disease have mechanistic similarities to diacetyl or 2,3-pentanedione?
- Can structure-activity relationships be developed that predict the airway toxicity of volatile compounds?
- Do inhalation-related and lung transplant-associated obliterative bronchiolitis 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 (or other systems' toxicity) for diacetyl and 2,3-pentanedione?
- What are the pathophysiological mechanisms of acute and chronic diacetyl or 2,3-pentanedione toxicity?
- What characteristics of butter flavoring powder contribute to airway injury?
- Can in vitro models of acute and chronic exposures to flavorings be developed to provide information useful to risk assessment?
- What is the relative respiratory toxicity of flavoring vapors compared to powders?
- Do mixed exposures of alpha-diketone flavorings have different airway epithelial toxicity from single agents in rodents?
- What laboratory tests are the best predictors of flavoring compounds that cause airway injury in humans?
- What is the role of diacetyl and other reactive carbonyl compounds in cigarette smoke (both tobacco cigarettes and e-cigarettes) in contributing to chronic obstructive pulmonary diseases?
- At what steady-state concentration of diacetyl, or above what cumulative exposure to diacetyl, does a fulminant, progressive pathological process initiate as opposed to a regular, constant, relatively lower rate of deterioration (as usually reflected in pulmonary function or structural changes)? Does this accelerated decline continue after cessation of exposure?

This page intentionally left blank.

Appendix A

OSHA PV2118 (Diacetyl)

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

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.

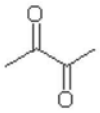
1.1.2 Toxic Effects[2] (This section is for information only and should not be taken as the basis of OSHA policy.)

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 H O
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 25ºC 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

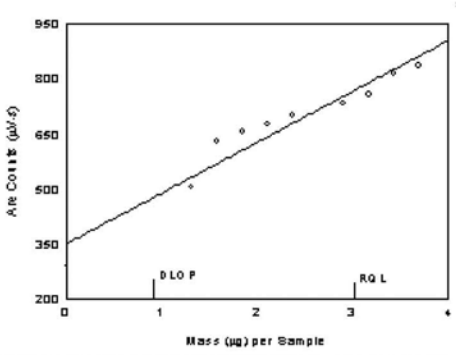


Figure 1.2.1 Plot of data to determine the DLOP/RQL for diacetyl. (Y=139X+349)

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.

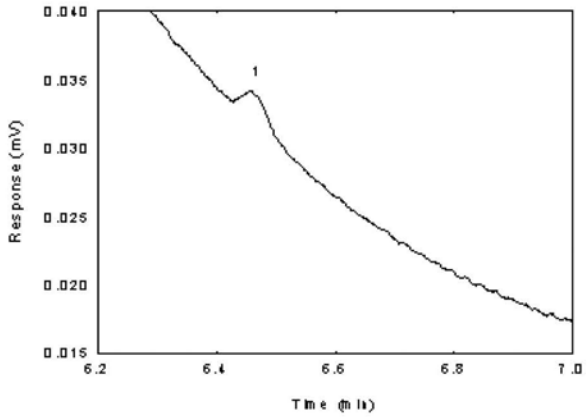


Figure 1.2.2 Chromatogram of the diacetyl standard near RQL (key: (1) diacetyl).

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)

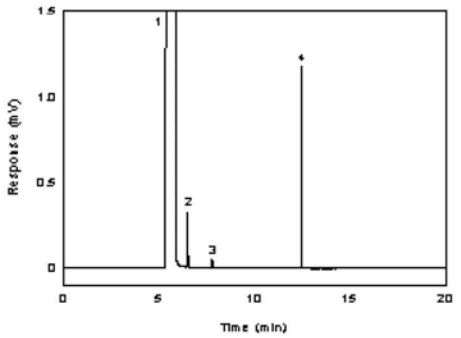


Figure 3.5.1 A chromatogram of 268 µg/ml diacetyl in 95:5 ethyl alcohol:water with 0.25 µl of p-cymene as internal standard. (Key: (1) ethyl alcohol, (2) diacetyl, (3) impurity, and (4) p-cymene).

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

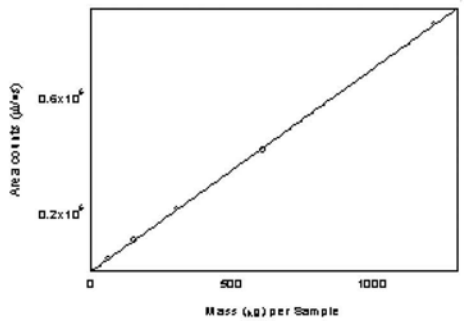


Figure 3.5.3 Calibration curve of diacetyl.
(Y = 696 x - 336)

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.

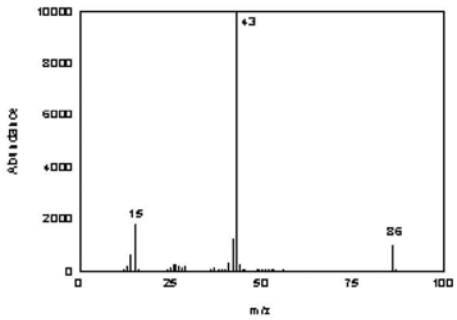


Figure 3.6.2 Mass spectrum of diacetyl.

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.

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.

This page intentionally left blank.

Appendix B

OSHA 1012 (Acetoin and Diacetyl)

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* *0.05 ppm (TWA) (0.18 mg/m^3) diacetyl*
OSHA PEL:	*none acetoin* *none diacetyl*
ACGIH TLV:	*none acetoin* *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)* *15 min at 0.2 L/min (3 L) (short term)*
Reliable quantitation limit:	*1.49 ppb (5.37 µg/m^3) acetoin* *1.30 ppb (4.57 µg/m^3) diacetyl*
Standard error of estimate at the target concentration:	*5.06% acetoin* *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)

---

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.

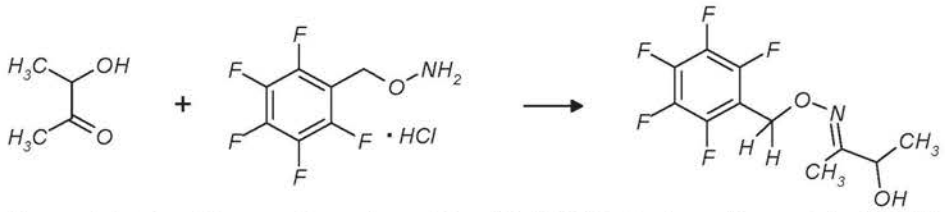


Figure 1.1.1.1. The reaction of acetoin with PFBHA to form the acetoin-PFBHA derivative.

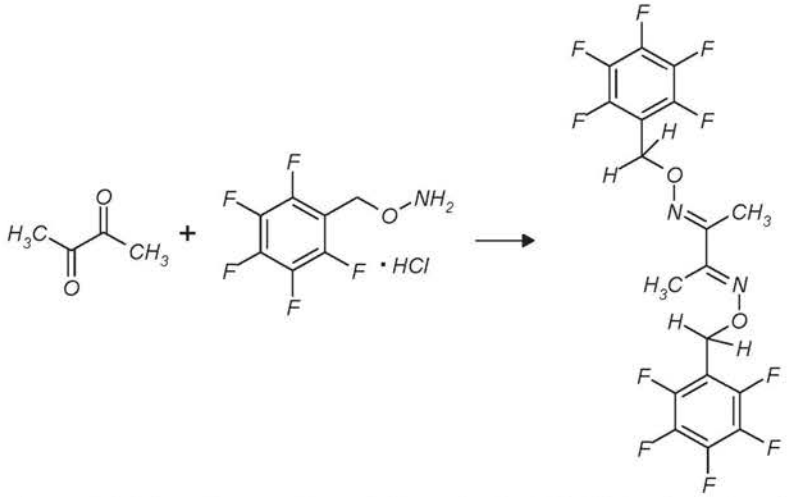


Figure 1.1.1.2. The reaction of diacetyl with PFBHA to form the diacetyl-PFBHA derivative.

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

---

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]

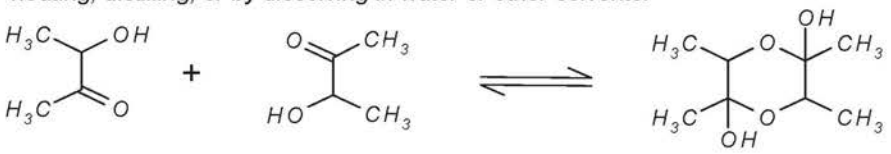
Acetoin is used as an aroma carrier and as a flavor ingredient to impart a creamy taste in fragrances and flavorings.[17] *Acetoin annual use in food and flavors manufacturing in 2004 was 34,000 pounds. Acetoin is used as a flavor ingredient for butter, milk, yogurt, and strawberry flavors. The FDA maximum 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.*

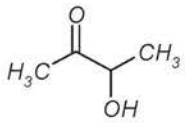
19 *Budavari, S., Ed; The Merck Index, 13th ed.; Merck & Co. Inc.: Whitehouse Station, NJ, 2001; p 68.*

20 *Material Safety Data Sheet: Acetoin, Chemwatch, Victoria, Australia (accesed 3/17/08).*

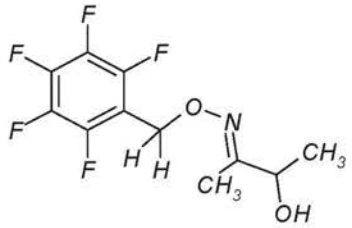
21 *Acetoin MSDS. SigmaAldrich Web site. http://www.sigmaaldrich.com/catalog/search/ProductDetail/ALDRICH/A17951 (accessed 3/17/2008).*

synonyms:	*acetyl methyl carbinol; 2,3-butanolone; 2-butanone, 3-hydoxy-; 2-butanol-3-one; dimethylketol; γ-hydroxy-β-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 °C (298 °F) (monomer)*
melting point:	*15 °C (59 °F) (monomer); 90 °C (194 °F) (dimer)*
density:	*1.005 g/mL @ 20/20 (monomer)*
molecular weight:	*88.11 (monomer)*
flash point:	*50.6 °C (123 °F) (closed cup) (monomer)*
autoignition temperature:	*370 °C (773.8 °F)*
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).*

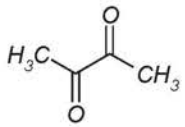
23 *CID: 90884 Acetyl Methyl Carbinol Dimer, 2008. Department of Health and Human Services, National Institutes of Health, National Center for Biotechnology Information. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=90884&loc=ec_rcs (accessed 3/17/2008).*

24 *Material Safety Data Sheet: Acetoin, 2008. The Good Scents Company Web site. http://www.thegoodscentscompany.com/msds/md102388.html (accessed 3/17/2008).*

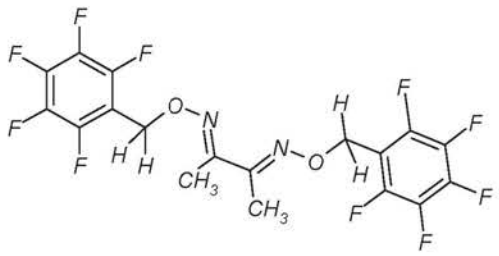
diacetyl[25,26,27,28]

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 °C (190 °F)*
melting point:	*3-4 °C (37.4-39.2 °F)*
density:	*0.99 g/mL @ 15/15*
molecular weight:	*86.09*
vapor pressure:	*7 kPa @ 20 °C*
flash point:	*26.7 °C (80 °F) (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 °C (545 °F)*

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 specially 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 °C. 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 °C. (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 °C. 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 °C. 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 °C. 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 23°C 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.*

---

[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:

GC conditions:

column:	*initial 100 °C, hold 1 min, program at 5 °C/min to 200 °C, hold 0 min*
injector:	*250 °C*
detector:	*250 °C*
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* *1.44 min PFBHA* *4.60 min 4-bromobenzylbromide* *5.04 min acetoin-PFBHA* *16.75 min diacetyl-PFBHA*

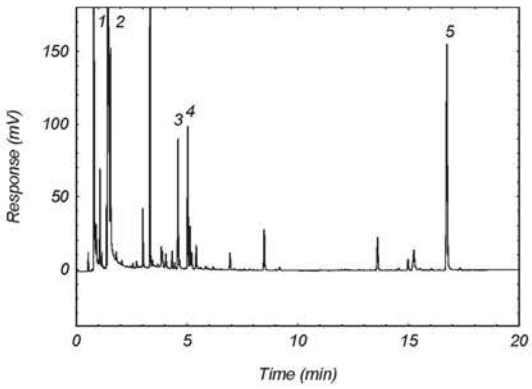


Figure 3.5.1. A chromatogram of the PFBHA derivatives of 1.60 µg/sample acetoin and 1.56 µg/sample diacetyl in the extraction solution. (Key: (1) ethyl alcohol; (2) PFBHA; (3) 4-bromobenzylbromide (ISTD); (4) acetoin-PFBHA; and (5) diacetyl-PFBHA; all other peaks are from PFBHA and its breakdown products)

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.

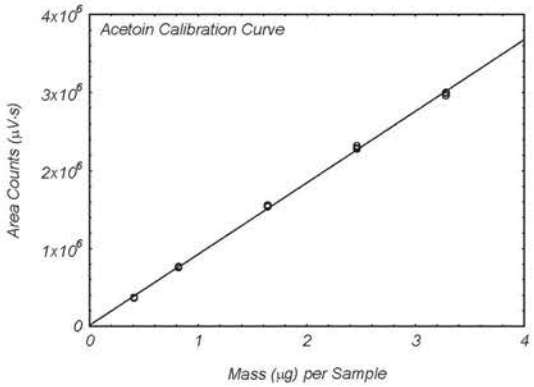


Figure 3.5.2.1. Calibration curve for acetoin. (y = 9.16E5x + 1.44E4)

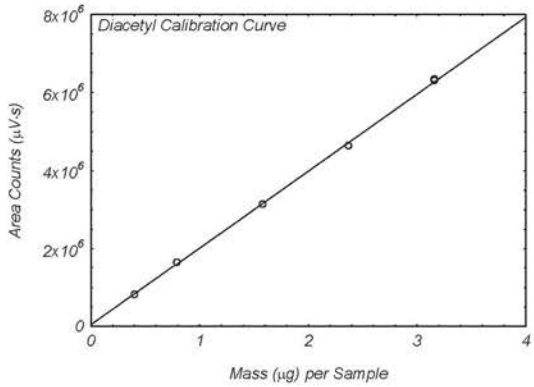


Figure 3.5.2.2. Calibration curve for diacetyl. (y = 1.97E6x + 4.59E4)

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
- $M_{front\ blank}$ is micrograms found on front blank tube
- M_{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

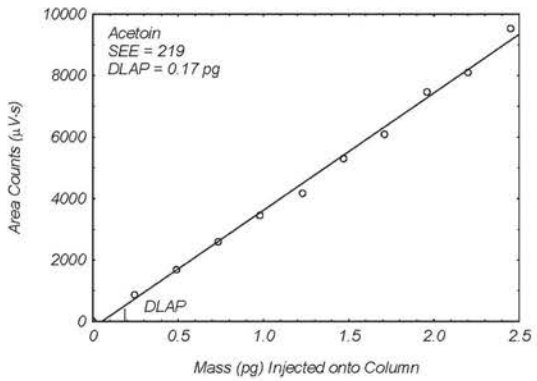


Figure 4.1.1. Plot of data to determine the DLAP for acetoin. (y = 3818x - 202)

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

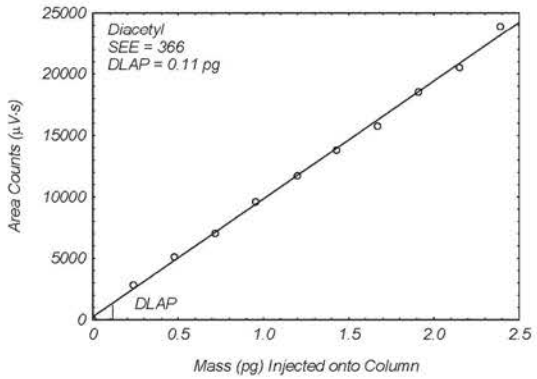


Figure 4.1.2. Plot of data to determine the DLAP for diacetyl. (y = 9595x + 238)

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

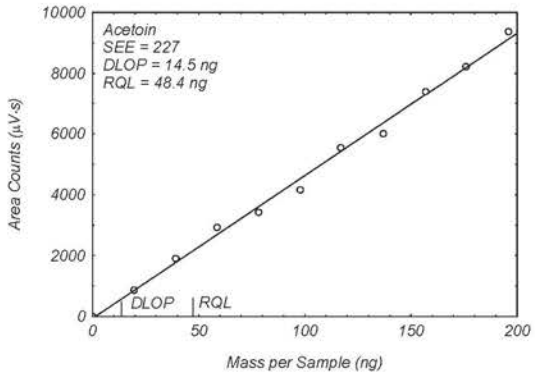


Figure 4.2.1. Plot of data to determine the DLOP/RQL for acetoin. (y = 46.9x - 63.1)

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

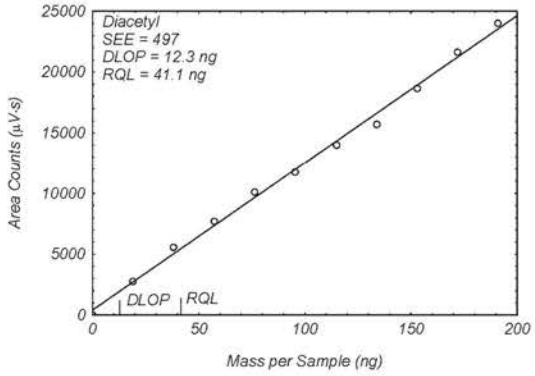


Figure 4.2.2. Plot of data to determine the DLOP/RQL for diacetyl. (y = 121x + 407)

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	$\mu g/m^3$	E_E
acetoin	48.4	1.49	5.37	102.3
diacetyl	41.1	1.30	4.57	97.3

E_E = extraction efficiency

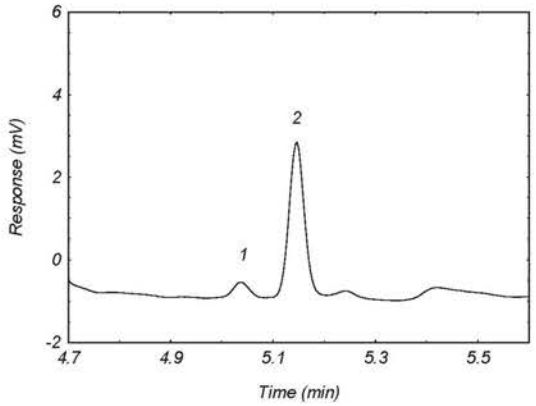


Figure 4.2.3. A chromatogram of the RQL of acetoin. (Key: (1) acetoin-PFBHA, (2) interference)

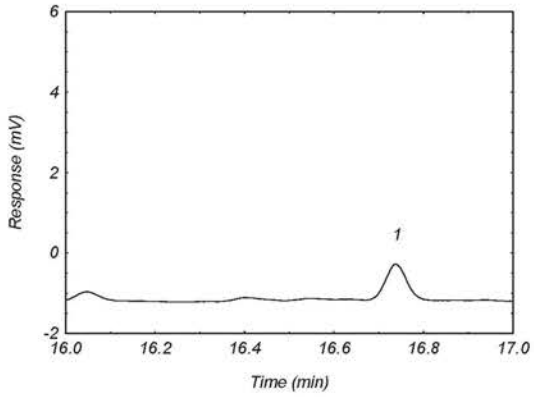


Figure 4.2.4. A chromatogram of the RQL of diacetyl. (Key: (1) diacetyl-PFHBA)

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 °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. 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

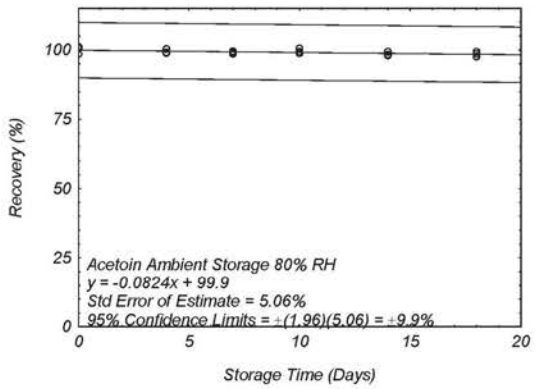


Figure 4.5.1. Ambient storage test for acetoin at 80% RH.

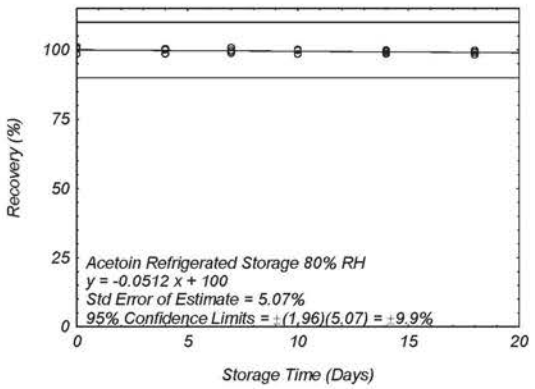


Figure 4.5.2. Refrigerated storage test for acetoin at 80% RH.

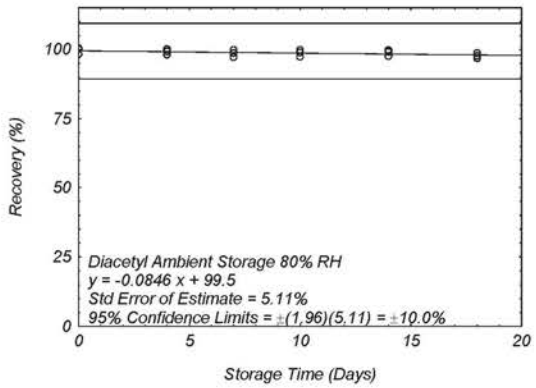


Figure 4.5.3. Ambient storage test for diacetyl at 80% RH.

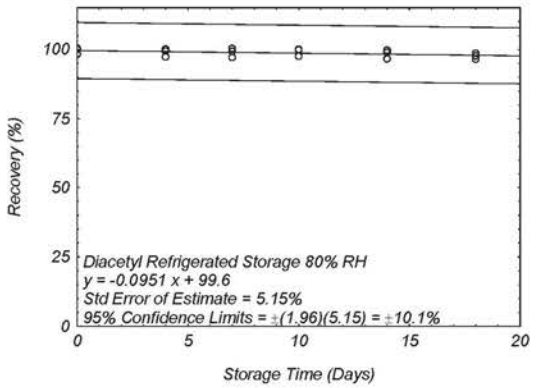


Figure 4.5.4. Refrigerated storage test for diacetyl at 80% RH.

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

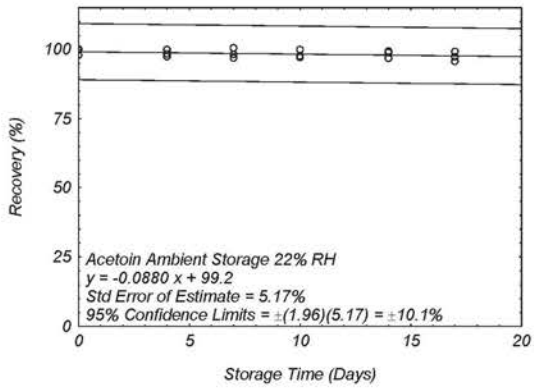


Figure 4.5.5. Ambient storage test for acetoin at 22% RH.

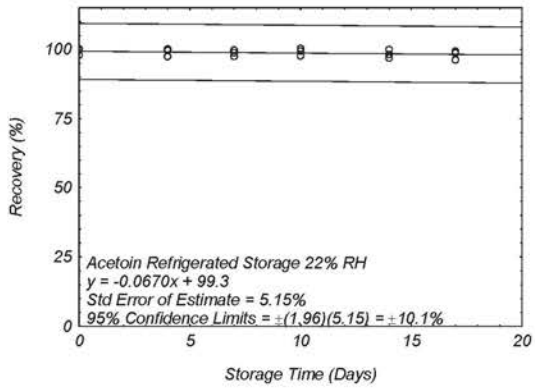


Figure 4.5.6. Refrigerated storage test for acetoin at 22% RH.

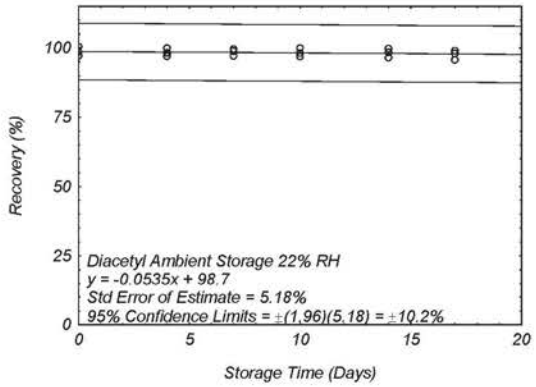


Figure 4.5.7. Ambient storage test for diacetyl at 22% RH.

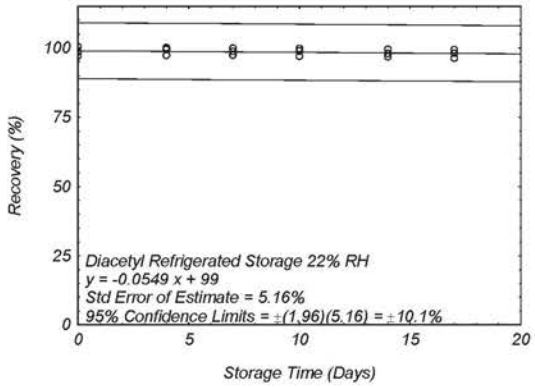


Figure 4.5.8. Refrigerated storage test for diacetyl at 22% RH.

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

	ambient		refrigerated	
day	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 23°C 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

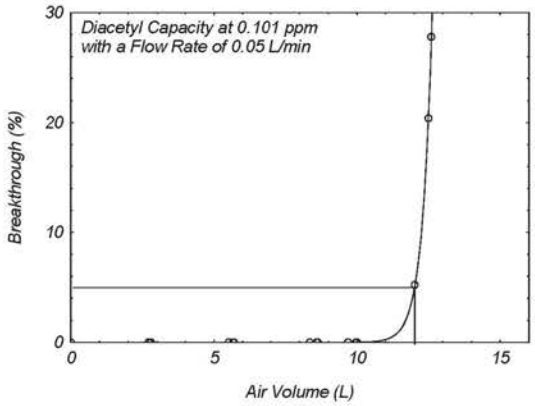


Figure 4.7.1. Five percent breakthrough test for diacetyl from a 0.101 ppm atmosphere, with a flow rate of 0.05 L/min.

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 °C at concentrations of 0.101 ppm (0.365 µg/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 °C. 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 °C. 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 °C. 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

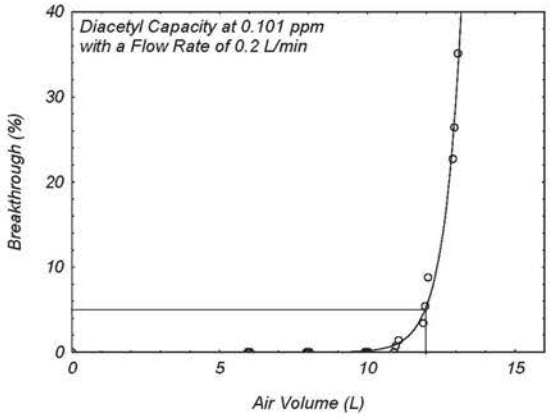


Figure 4.7.2. Five percent breakthrough test for diacetyl from a 0.101 ppm atmosphere, with a flow rate of 0.2 L/min.

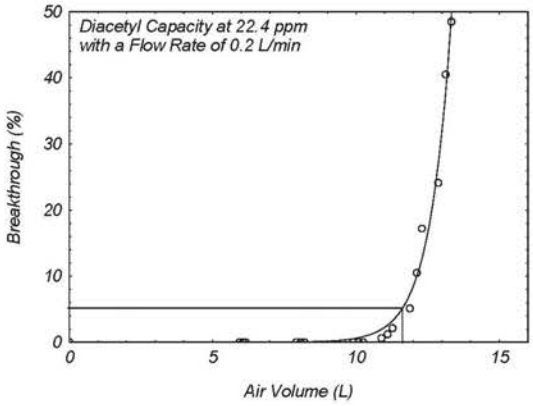


Figure 4.7.3. Five percent breakthrough test for diacetyl from a 22.4 ppm atmosphere, with a flow rate of 0.2 L/min.

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 °C 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 4.8.4
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 4.8.5
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.

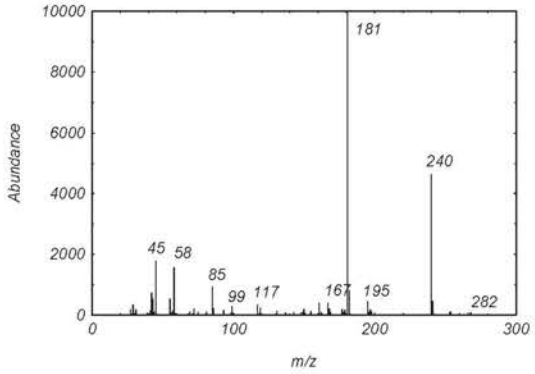


Figure 4.10.1. Mass spectrum of acetoin-PFBHA derivative.

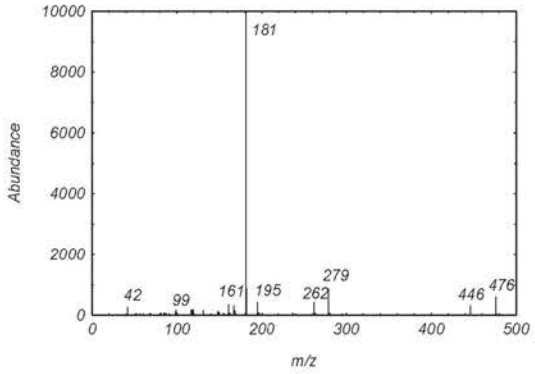


Figure 4.10.2. Mass spectrum of diacetyl-PFBHA derivative.

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 × 30-cm) from the vapor generator, and then into a glass exposure chamber (76-cm × 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.

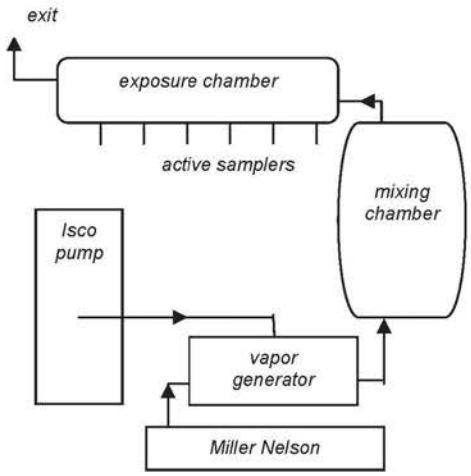


Figure 4.11. The test atmosphere generation and sampling apparatus.

This page intentionally left blank.

Appendix C

Acetoin Diacetyl 1013

Acetoin
Diacetyl

Method no.:	1013
Control no.:	T-1013-FV-01-0809-M
Target concentration:	0.5 ppm (1.80 mg/m^3) acetoin 0.5 ppm (1.76 mg/m^3) diacetyl
OSHA PEL:	none for acetoin none for diacetyl
ACGIH TLV:	none for acetoin 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) 15 min at 0.2 L/min (3 L) (short term)
Reliable quantitation limit:	0.011 ppm (0.039 mg/m^3) acetoin 0.012 ppm (0.041 mg/m^3) diacetyl
Standard error of estimate at the target concentration:	5.7% acetoin 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

Michael Simmons
Warren Hendricks

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]

---

[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]

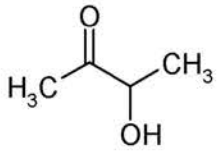
Acetoin occurs as the liquid monomer and the solid dimer. The monomer can be formed from the dimer by dissolving in water or other solvents.

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 °C (298 °F) @ 760 mmHg (monomer)
melting point:	15 °C (59 °F) (monomer); 91 °C (196 °F) (dimer)
density:	1.005 (g/mL@ 25 °C) (monomer)
molecular weight:	88.11 (monomer)
flash point:	46.7 °C (116 °F) (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)

---

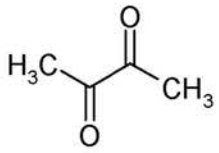
[14] Burdock, G. A. *Fenaroli's Handbook of Flavor Ingredients*, 5th ed.; CRC Press: Boca Raton, FL, 2005; pp 11-12.
[15] Burdock, G. A. *Fenaroli's Handbook of Flavor Ingredients*, 5th ed.; CRC Press: Boca Raton, FL, 2005; pp 411-412.
[16] Kanwal, R.; Boylstein, R. J.; Piacitelli, C. NIOSH Health Hazard 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 °C (190 °F)
melting point:	3-4 °C (37.4-39.2 °F)
density:	0.99 (g/mL@ 15/15)
molecular weight:	86.09
vapor pressure:	7 kPa @ 20 °C
flash point:	26.7 °C (80 °F) (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 °C (545 °F)
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 °C 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) 8.1 min (acetoin) 7.5 min (ISTD)

FID conditions

hydrogen flow:	40 mL/min
air flow:	450 mL/min
nitrogen makeup flow:	45 mL/min

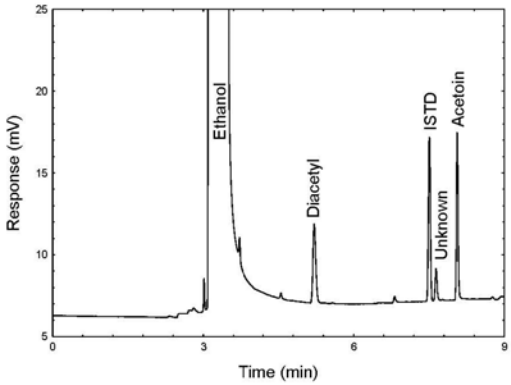


Figure 3.5.1. Chromatogram obtained at target concentrations with recommended conditions.

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.

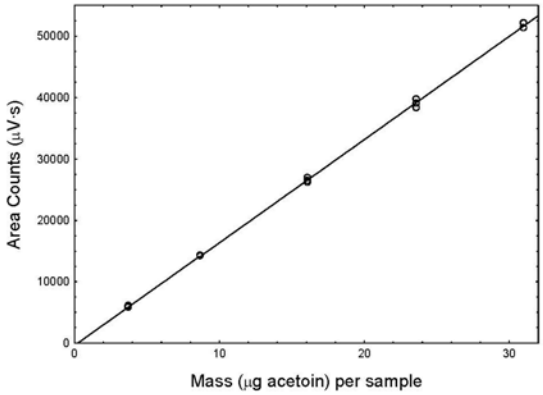


Figure 3.5.2.1. Calibration curve of acetoin. (Y = 1678X – 389)

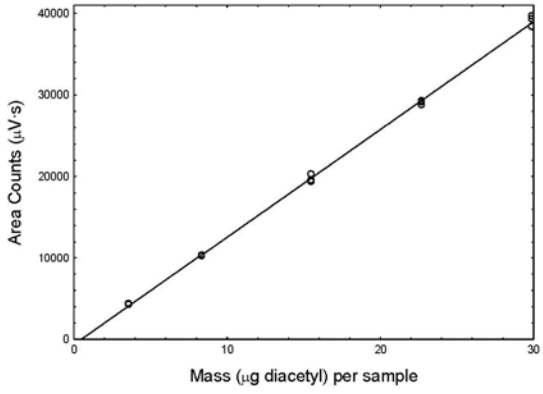


Figure 3.5.2.2. Calibration curve of diacetyl. (Y = 1320X – 625)

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

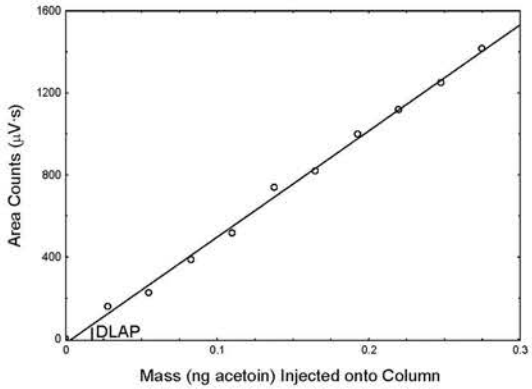


Figure 4.1.1. Plot of data to determine the DLAP for acetoin. (Y = 5171X – 19.9)

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

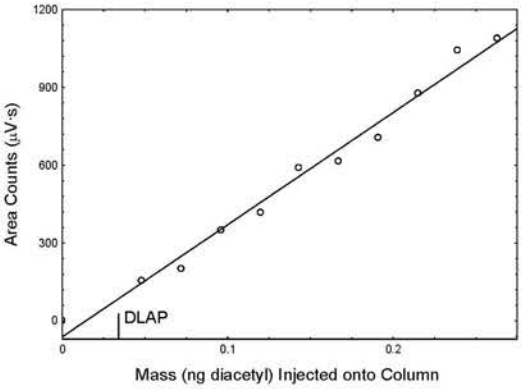


Figure 4.1.2. Plot of data to determine the DLAP for diacetyl. (Y = 4325X - 62)

[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

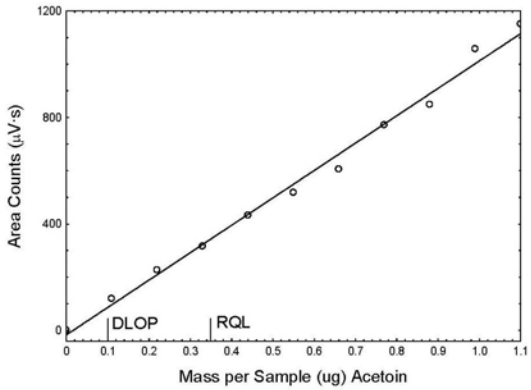


Figure 4.2.1. Plot of data to determine the DLOP/RQL for acetoin. (Y = 1029X – 16.8)

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

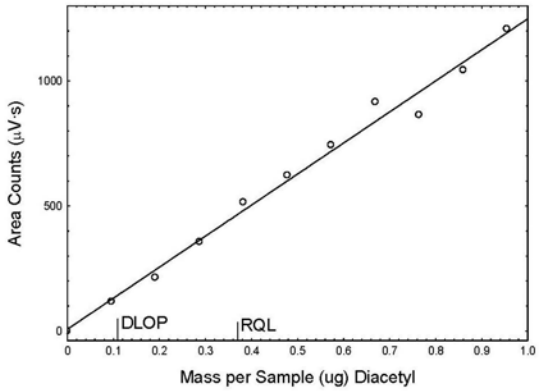


Figure 4.2.2. Plot of data to determine the DLOP/RQL for diacetyl. (Y = 1241X – 7.3)

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%.

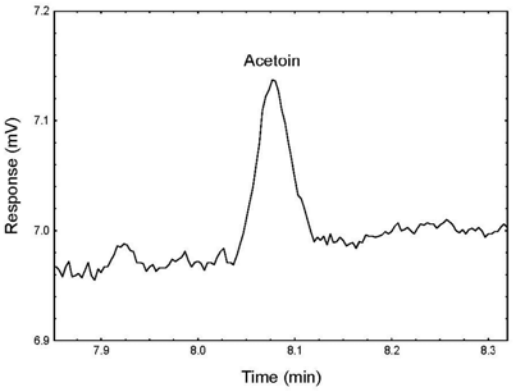


Figure 4.2.3. Chromatogram of acetoin at the RQL.

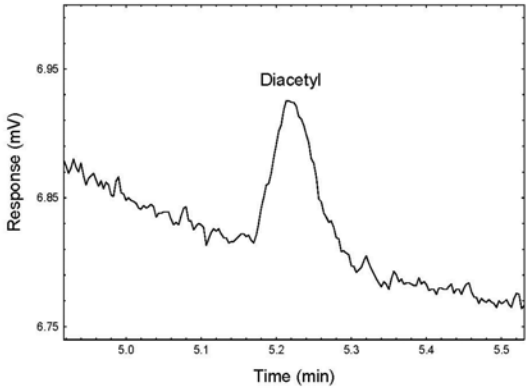


Figure 4.2.4. Chromatogram of diacetyl at the RQL.

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

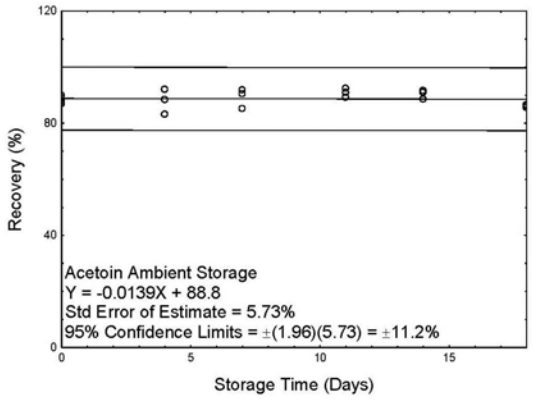


Figure 4.5.1. Ambient storage test for acetoin.

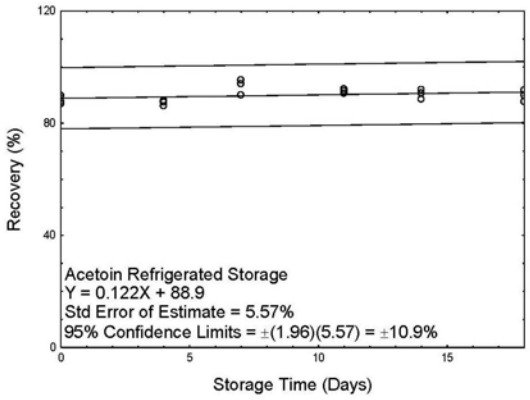


Figure 4.5.2. Refrigerated storage test for acetoin.

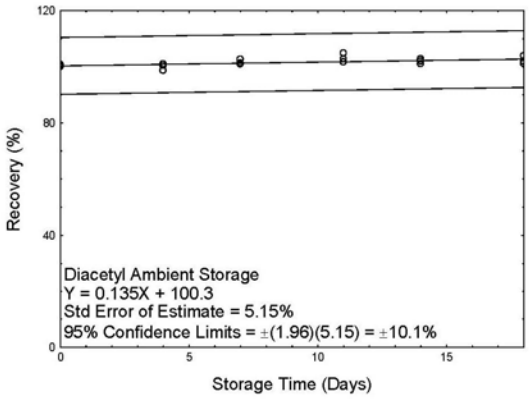


Figure 4.5.3. Ambient storage test for diacetyl.

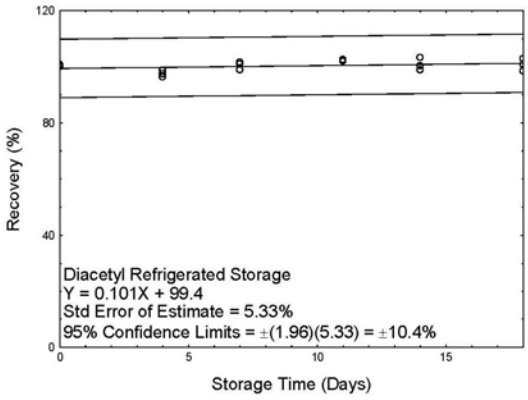


Figure 4.5.4. Refrigerated storage test for diacetyl.

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

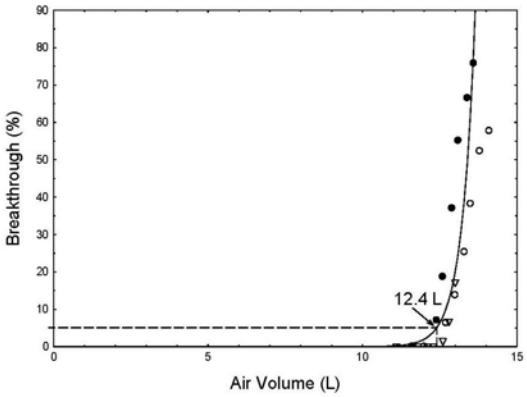


Figure 4.7. Five percent breakthrough air volume for diacetyl.

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%.

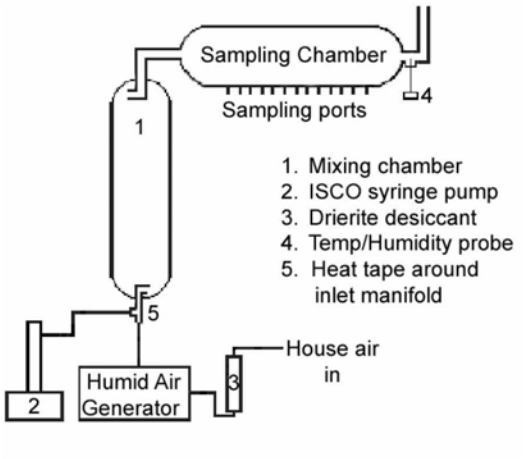


Figure 4.11. Diagram of apparatus used to generate test atmospheres.

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.

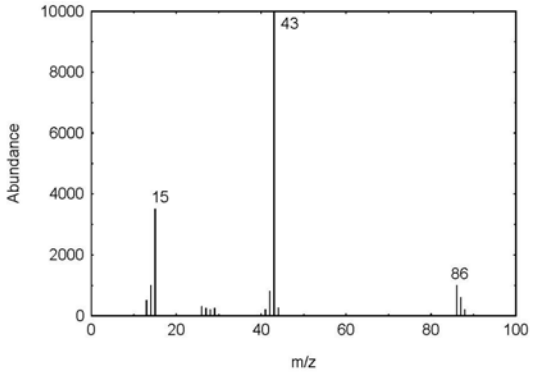


Figure 4.12.1. Mass spectrum of diacetyl.

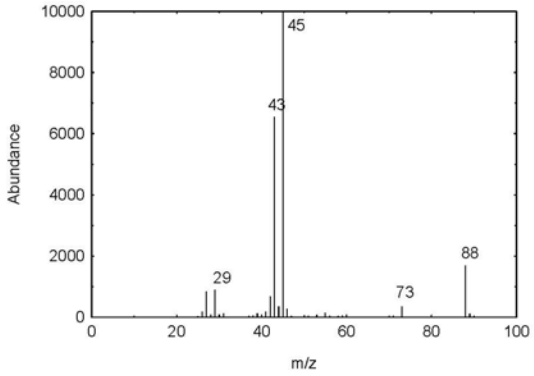


Figure 4.12.2. Mass spectrum of acetoin.

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).

Appendix D

2, 3-Pentanedione

2,3-Pentanedione

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) 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

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. 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.

---

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

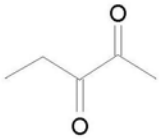
2,3-Pentanedione is a natural flavorant and odorant that is also synthesized for use in odor and flavor manufacturing.[6] It is used to give products a buttery, nutty, cheesy, fruity, toasted, chocolate, or caramel taste. It also gives products a buttery, fruity, and caramel odor. There can be as much 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



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).

[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 13.5 min 3-pentanone

FID conditions:

hydrogen flow:	40 mL/min
air flow:	450 mL/min
nitrogen makeup flow:	40 mL/min

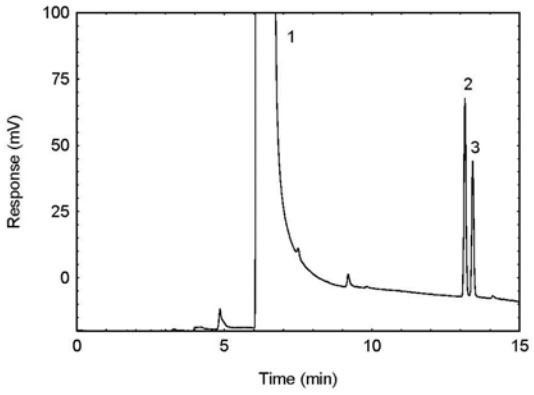


Figure 3.5.1.1. A chromatogram of 20.4 µg/sample 2,3-pentanedione. (Key: 1) ethyl alcohol; 2) 2,3-pentanedione; and 3) 3-pentanone.)

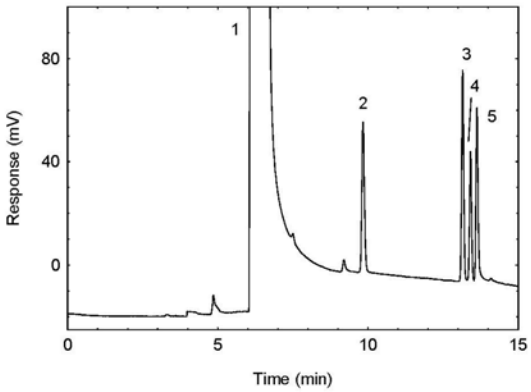


Figure 3.5.1.2. A chromatogram of 20.4 µg/sample 2,3-pentanedione, 15.8 µg/sample acetoin, and 15.6 µg/sample diacetyl. (Key: 1) ethyl alcohol; 2) diacetyl; 3) 2,3-pentanedione; 4) 3-pentanone; and 5) acetoin.)

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.

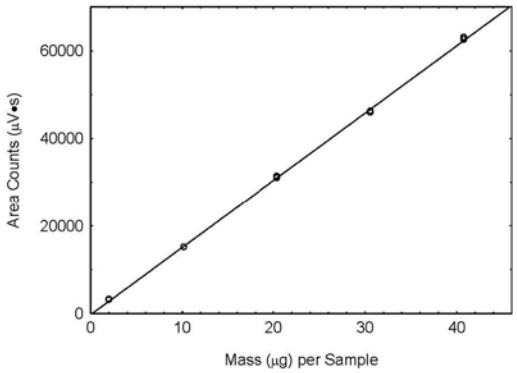


Figure 3.5.2.1. Calibration curve for 2,3-pentanedione.
(y = 1535x - 295)

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

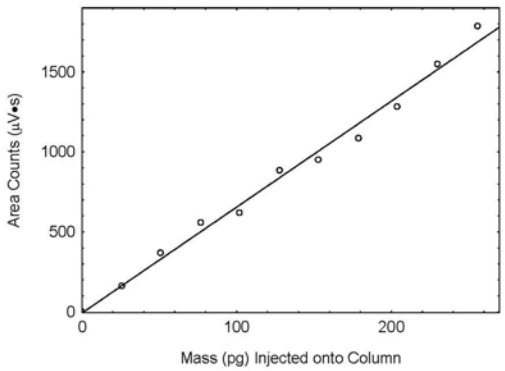


Figure 4.1. Plot of data to determine the DLAP (y = 6.62x - 7.12).

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

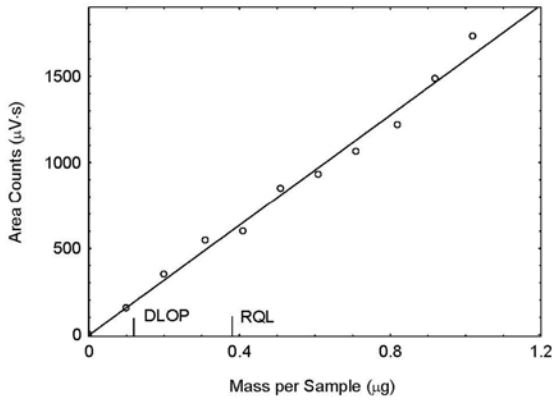


Figure 4.2.1. Plot of data to determine the DLOP/RQL (y = 1597x – 3.74).

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.

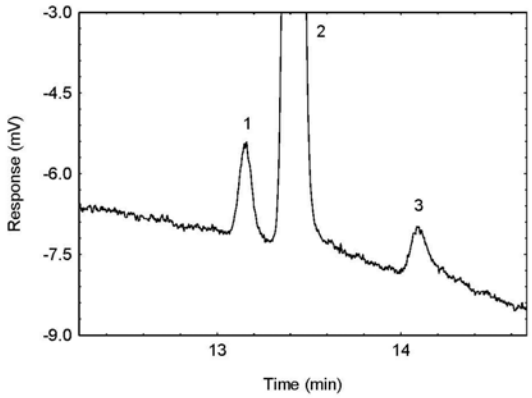


Figure 4.2.2. A chromatogram of the RQL of 2,3-pentanedione. (Key: 1) 2,3-pentanedione; 2) 3-pentanone; and 3) interferant.)

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

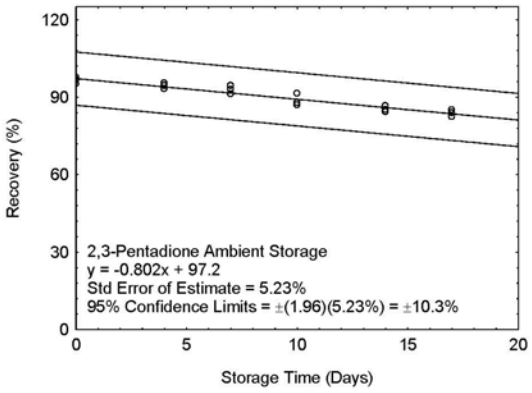


Figure 4.4.1. Ambient storage test for 2,3-pentanedione.

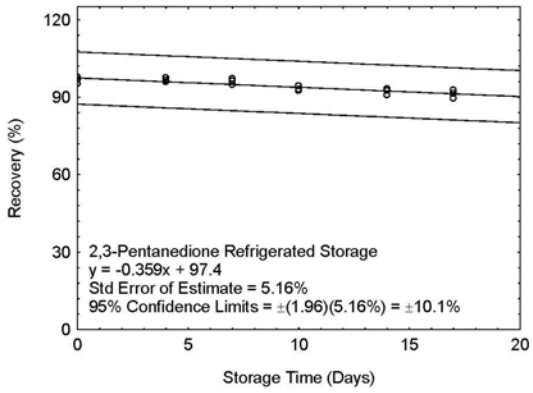


Figure 4.4.2. Refrigerated storage test for 2,3-pentanedione.

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

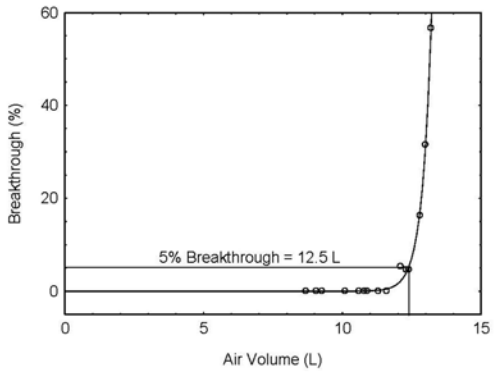


Figure 4.7. Five percent breakthrough air volume for 2,3-pentanedione.

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 23°C 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.

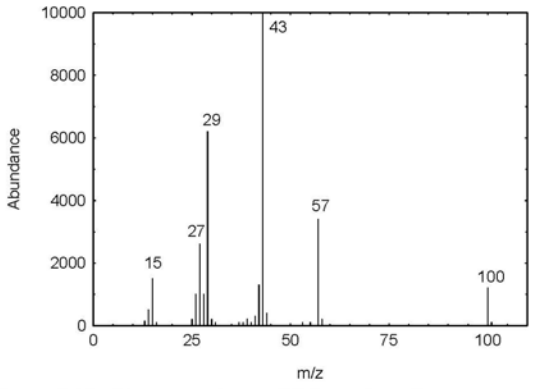


Figure 4.10. Mass spectrum of 2,3-pentanedione.

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.

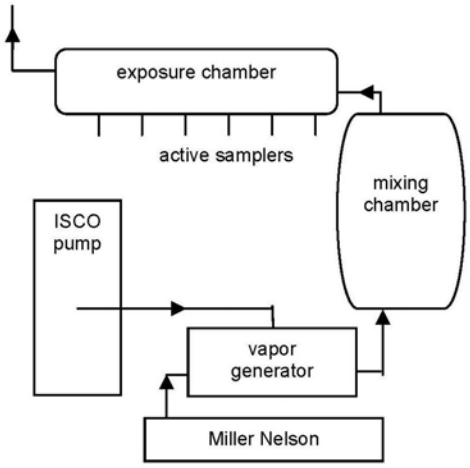


Figure 4.11. The test atmosphere generation and sampling apparatus.

Appendix E

Volatile Organic Compounds (Screening) 2549

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 ($\hat{S}_r$):	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

NIOSH Manual of Analytical Methods (NMAM), Fourth Edition, 5/15/96

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

NIOSH Manual of Analytical Methods (NMAM), Fourth Edition, 5/15/96

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:

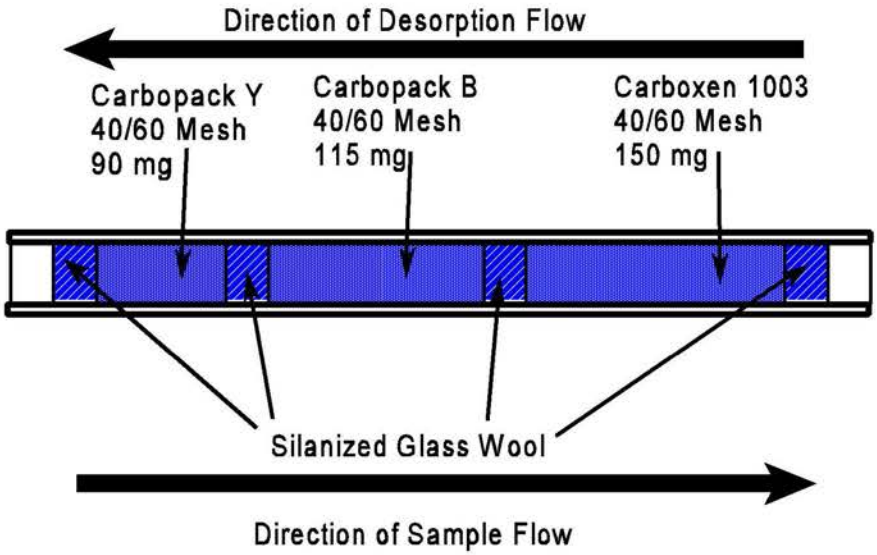


Figure 1

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.

This page intentionally left blank.

Appendix F

Correcting Diacetyl Concentrations from Air Samples Collected with NIOSH Method 2557

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

Jean Cox-Ganser,[1] Gary Ganser,[2] Rena Saito,[1] Gerald Hobbs,[3] Randy Boylstein,[1] Warren Hendricks,[4] Michael Simmons,[4] Mary Eide,[4] Greg Kullman,[1] and Chris Piacitelli[1]

[1]Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Morgantown, West Virginia
[2]Department of Mathematics, West Virginia University, Morgantown, West Virginia
[3]Department of Statistics, West Virginia University, Morgantown, West Virginia
[4]Salt Lake Technical Center, Occupational Safety and Health Administration, Sandy, Utah

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

Correspondence to: Jean Cox-Ganser, Field Studies Branch, Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, 1095 Willowdale Road, MS 2800, Morgantown, WV 26505–2888; e-mail: jjc8@cdc.gov.

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.).

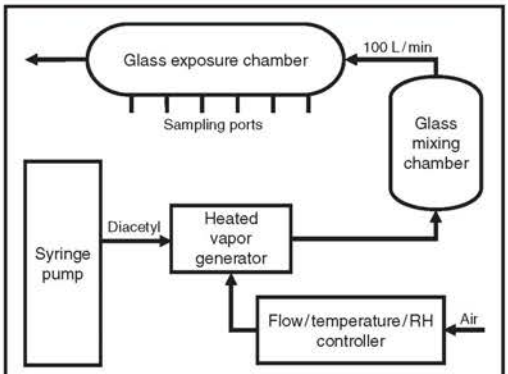


FIGURE 1. Test atmosphere generation apparatus.

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

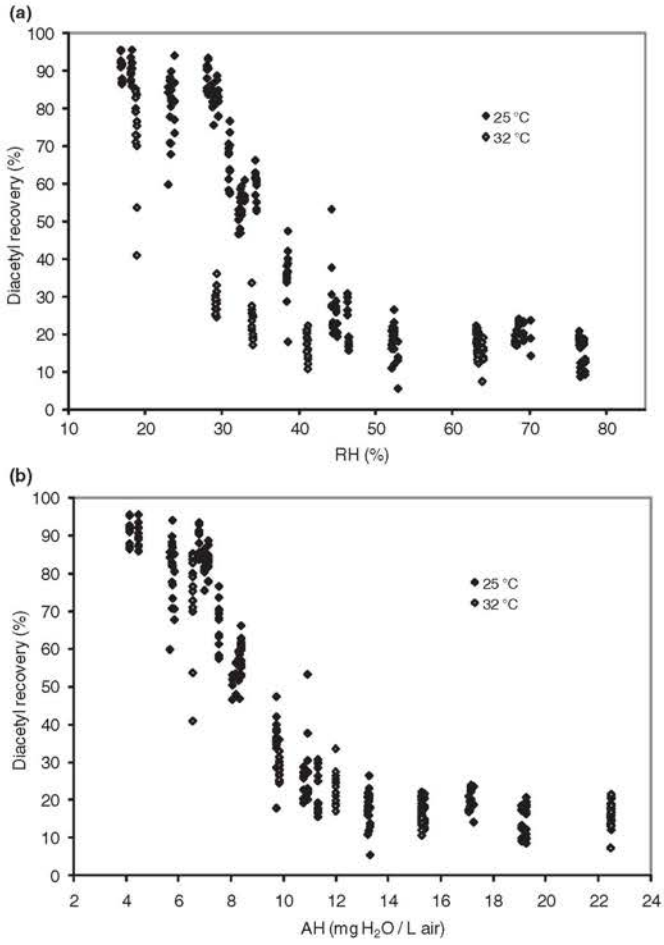


FIGURE 2. Plots for 5.0 ppm target diacetyl concentration data from 25°C and 32°C showing diacetyl recovery in terms of (a) RH and (b) AH.

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]

$$AH = \frac{13.25\,RH \exp\left(\frac{17.67\,T_c}{T_c + 243.50}\right)}{T_c + 273.15} \quad (1)$$

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 θ_1, θ_2, θ_3, and θ_4, each of which has graphical meaning. The parameter θ_1 represents the horizontal asymptote on the right-hand side of the graph where humidity is at the highest level; θ_2 represents the horizontal asymptote on the left-hand side of the graph where humidity is at the lowest level; θ_3 is the "slope" or the shape parameter; and θ_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:

$$Y = \theta_1 + \frac{\theta_2 - \theta_1}{1 + \exp[\theta_3(X - \theta_4)]} \quad (2)$$

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 θ_1 was well approximated ($R^2 = 0.99$) by a linear function of target concentration C_0

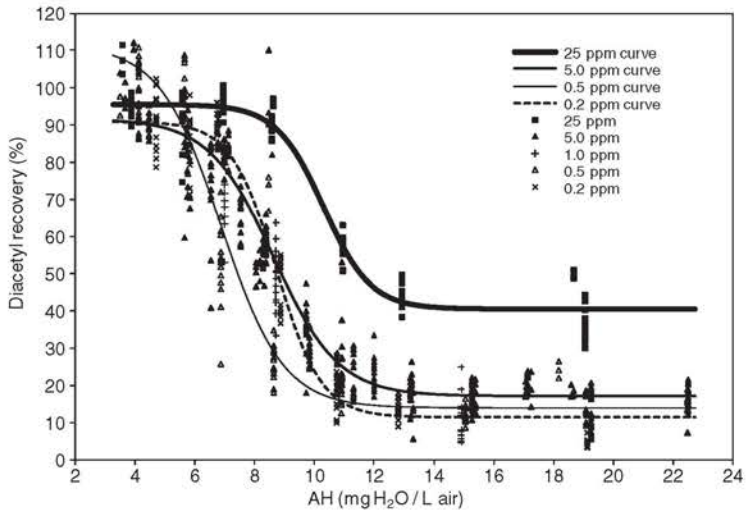


FIGURE 3. Four-parameter logistic models fitted to test data. Note: Data are insufficient to create a logistic curve for 1.0 ppm.

TABLE II. Parameter Coefficients for the Overall Diacetyl Correction Equation and for Two Sampling Flow Rates

Parameter	Overall	Sampling Flow Rate	
		50 cc/min	150 cc/min
b_0	6.91166	5.85971	8.32618
m_1	1.69272	1.70372	1.68917
θ_2	101.31390	101.06329	101.81000
θ_3	0.72068	0.70943	0.73539
θ_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 θ_2, θ_3, and θ_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, θ_2, θ_3, and θ_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

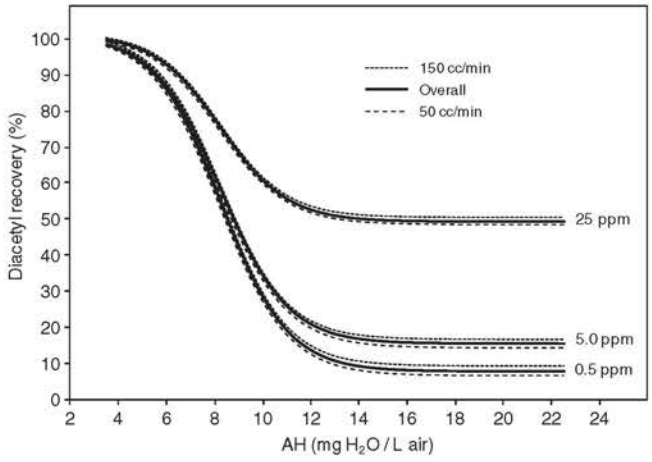


FIGURE 4. Model for data from samples extracted on Day 1 after sampling (Eq. 3) applied at three diacetyl concentrations using parameter coefficients (b_0, m_1, θ_2, θ_3, θ_4) for the whole data set (overall) and the two sampling flow rates used in the experiment.

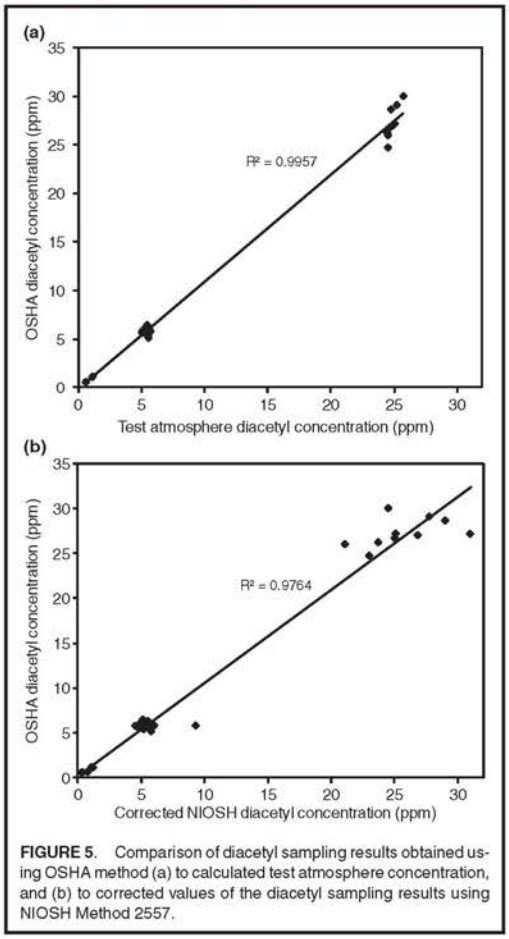


FIGURE 5. Comparison of diacetyl sampling results obtained using OSHA method (a) to calculated test atmosphere concentration, and (b) to corrected values of the diacetyl sampling results using NIOSH Method 2557.

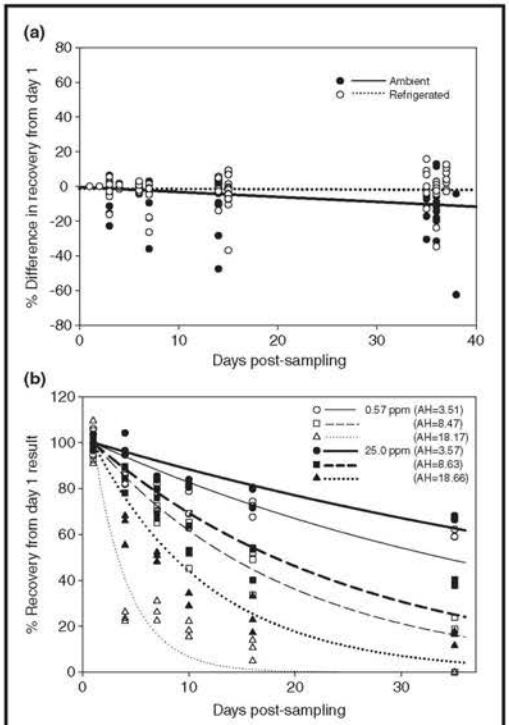


FIGURE 6. (a) Extract stability: Samples extracted and analyzed on Day 1 post-sampling and the remaining sorbent material and extract kept at ambient or refrigerated temperature before further analyses on subsequent days. Lines represent regressions. (b) In-tube stability: Samples extracted and analyzed on the same day. Curves were created from Eq. 4.

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.

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:

$$\text{Normalized recovery} = g(C_0, AH, t) = \exp[-(f_1(C_0) + f_2(C_0)AH + f_3(C_0)AH^2)(t-1)] \quad (4)$$

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:

$$\text{Percent recovered diacetyl} = \frac{100c}{C_0} = h(C_0, AH)g(C_0, AH, t) \quad (5)$$

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:

$$aC_0^2 + bC_0 - \left(\frac{c}{g(C_0, AH, t)}\right) = 0 \quad (6)$$

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:

$$C_0 = \frac{-b + \sqrt{b^2 + 4a\left(\frac{c}{g(C_0, AH, t)}\right)}}{2a} \quad (7)$$

(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 $C_0^{(i+1)}$

$$C_0^{(i+1)} = \frac{-b + \sqrt{b^2 + 4a\left(\frac{c}{g(C_0^{(i)}, AH, t)}\right)}}{2a} \quad (8)$$

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:

$$C_0^{(i+1)} = \frac{c}{g\left(C_0^{(i)}, AH, t\right)} \quad (9)$$

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

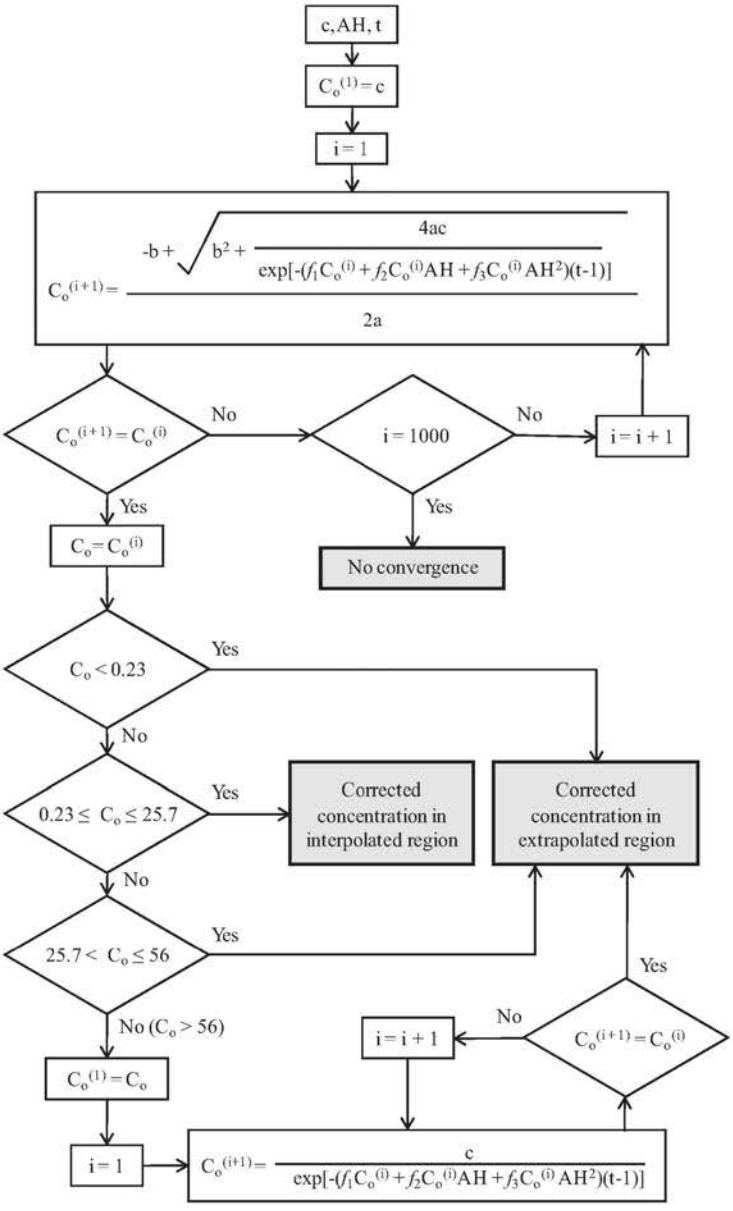


FIGURE 7. Flow diagram of the correction procedure that begins with known values of recovered diacetyl concentration (c), absolute humidity (AH) during sampling, and the number of days from sampling to extraction (t), and ends with the corrected diacetyl concentration in either the interpolated or extrapolated region. As explained in the text, there are some conditions of no convergence.

Journal of Occupational and Environmental Hygiene February 2011 67

Appendix 2 Page 9

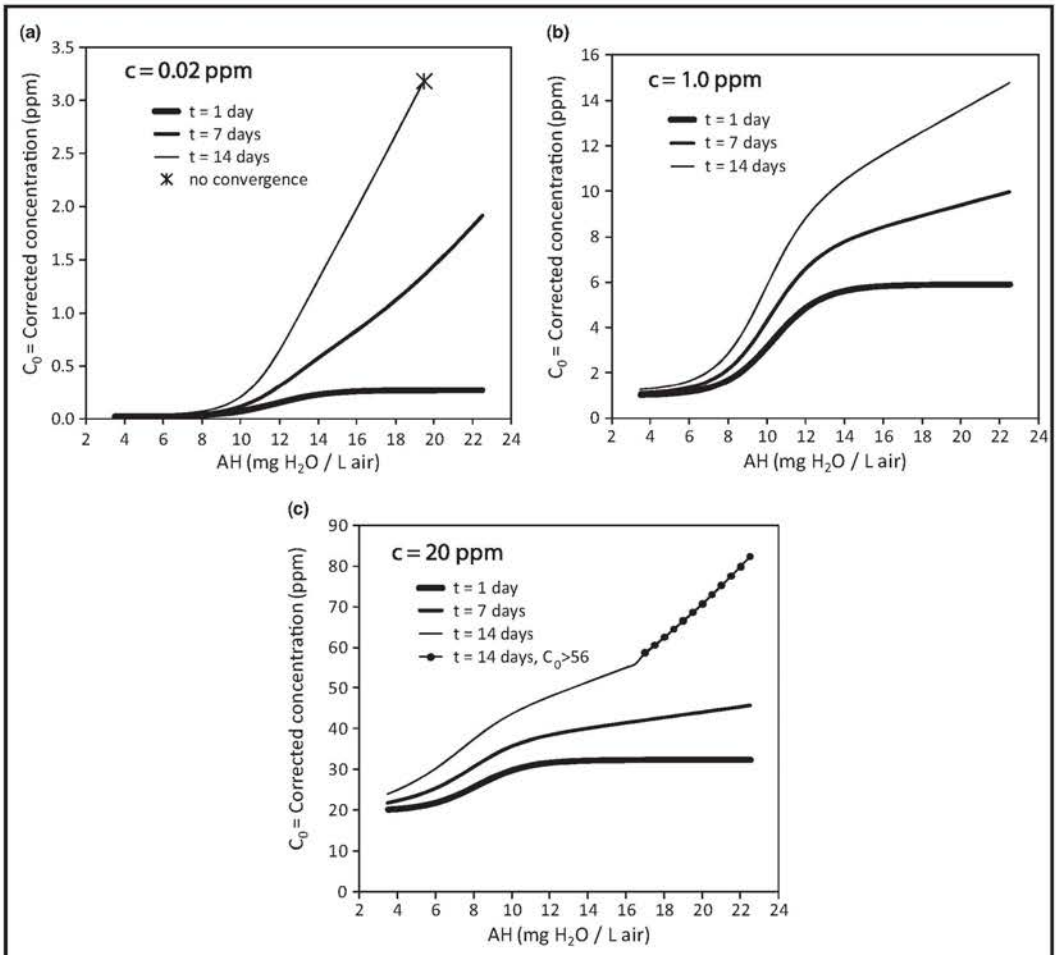


FIGURE 8. Predicted diacetyl concentrations using the model at selected laboratory-reported (recovered) concentrations of (a) 0.02 ppm, (b) 1.0 ppm, and (c) 20 ppm.

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.

REFERENCES

1. **Kreiss, K., A. Gomaa, G. Kullman, et al.:** Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. *N. Engl. J. Med. 347*:330–338 (2002).
2. **Kanwal, R.:** Bronchiolitis obliterans in workers exposed to flavoring chemicals. *Curr. Opin. Pulm. Med. 14*:141–146 (2008).
3. **Van Rooy, F.G., J.M. Rooyackers, M. Prokop, et al.:** Bronchiolitis obliterans syndrome in chemical workers producing diacetyl for food flavorings. *Am. J. Respir. Crit. Care Med. 176*(5):498–504 (2007).
4. **Hubbs, A., L. Battelli, R. Mercer, et al.:** Inhalation toxicity of the flavoring agent, diacetyl (2,3-butanedione), in the upper respiratory tract of rats. *Toxicol. Sci. 78*(*Suppl 1*): 438–439 (2004).
5. **Hubbs, A.F., W.T. Goldsmith, M.L. Kashon, et al.:** Respiratory toxicologic pathology of inhaled diacetyl in Sprague-Dawley rats. *Toxicol. Pathol. 36*:330–344 (2008).
6. **Pendergrass, S.M.:** Method development for the determination of diacetyl and acetoin at a microwave popcorn plant. *Environ. Sci. Technol. 38*(*3*):858–861 (2004).
7. "Diacetyl: Method 2557. NIOSH Manual of Analytical Methods (NMAM)." [Online] Available at http://www.cdc.gov/niosh/nmam/pdfs/2557.pdf (Accessed May 21, 2010).
8. **McKernan, L.T., E. Burroughs, J. Deddens, et al.:** Analytical performance criteria: Field evaluation of diacetyl sampling and analytical methods. *J. Occup. Environ. Hyg. 5*:D111–D116 (2008).
9. "Diacetyl: Method PV2118. OSHA Sampling and Analytical Methods." [Online] Available at http://www.osha.gov/dts/sltc/methods/partial/t-pv2118/t-pv2118.html (Accessed May 21, 2010).
10. "Diacetyl: Method 1013. OSHA Sampling and Analytical Methods." [Online] Available at http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (Accessed May 21, 2010).
11. **Bolton, D.:** The computation of equivalent potential temperature. *Mon. Wea. Rev. 108*:1046–1053 (1980).

This page intentionally left blank.

Appendix G

JEM Tables for Four Plants

JEM Tables for Four Plants

The mean diacetyl vapor concentrations estimated for the three plants with a single NIOSH survey are shown in Tables A3.1 – A3.3. For the fourth plant, Company G, time-dependent exposure levels were estimated in the NIOSH-OSHA JEM collaboration (Table A3.4). All Method 2557 measurements were corrected for temperature, humidity, and days to extraction.

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	Line packer, line stacker on both lines	1	1.358	7/1/1986	2/11/2001
Polypropylene		2	0.346	2/12/2001	5/21/2001

Production				
	3	0.182	5/22/2001	9/6/2002
	4	0.012	9/7/2002	8/15/2003

Appendix H

Development of a Job Exposure Matrix for Company G

1.0 Development of a Job Exposure Matrix for Company G

1.1 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 vapor in parts per million parts air (ppm). This JEM includes estimates for eight 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 are described below.

1.2 Industrial Hygiene Surveys

A total of nine industrial hygiene surveys were conducted over a period of 4 years from 2000 to 2003. The sampling was typically conducted during the day shift, because this shift presented the opportunity to sample all job categories. However, samples were also collected from second and third shifts, but not routinely. The dates for these industrial hygiene surveys are presented in Table A4.1.

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 eight 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.

1.3 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 A4.2 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 subsequent surveys (i.e. surveys 2-9). 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 2–9, 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 2–9, 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 first survey is described in Table A4.3.

1.4 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 eight job categories to reflect impact of the exposure control changes implemented at the plant between November 2000 to July 2003. Table A4.4 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 A4.5. 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.

1.5 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 onward, 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. Because 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.

References

Corn M, Esmen NA [1979]. Workplace exposure zones for classification of employee exposures to physical and chemical agents. Am Ind Hyg Assoc J *40*(1):47–57.

Cox-Ganser J, Ganser G, Saito R, Hobbs G, Boylstein R, Hendricks W, Simmons M, Eide M, Kullman G, Piacitelli C [2011]. Correcting diacetyl concentrations from air samples collected with NIOSH method 2557. J Occup Environ Hyg *8*(2):59-70.

Ganser GH, Hewett P [2010]. An accurate substitution method for analyzing censored data. J Occup Environ Hyg *7*(4):233–244.

Kreiss K, Gomaa A, Kullman G, Fedan K, Simoes EJ, Enright PL [2002]. Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. N Engl J Med *347*(5):330–338.

Kullman G, Boylstein R, Jones W, Piacitelli C, Pendergrass S, Kreiss K [2005]. Characterization of respiratory exposures at a microwave popcorn plant with cases of bronchiolitis obliterans. J Occup Environ Hyg *2*(3):169–178.

NIOSH [2004]. NIOSH Respirator Selection Logic. By Bollinger N. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2005–100.

NIOSH [2006]. Hazard evaluation and technical assistance report: Gilster-Mary Lee Corporation, Jasper, MO. By Kanwal R, Kullman G, Fedan K, Kreiss K. Cincinnati, OH: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, NIOSH HETA Report No. 2000–0401–2991.

Smith TJ [1992]. Occupational exposure and dose over time: limitations of cumulative exposure. Am J Ind Med *21*(1):35–51.

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 from surveys 2-9 and apply model to survey 1 area samples to determine personal equivalent diacetyl exposures.
6	Microwave Packaging Line	Model personal to area diacetyl concentrations from surveys 2-9 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 2-9 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)	
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)

8

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:** 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. 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:** 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 microwave mixing room. 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. 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.

	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:** 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:** 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. 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:** Time 1 (Survey 1 & 2 sampling results): Reflects exposures before major control changes that would impact microwave mixer exposures. 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. 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. 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:** Time 1 (Surveys 1 & 2 sampling results): Reflects exposures before major control changes that would impact microwave line exposures. 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. 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. 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.
7	**Bag Printing:** 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. 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. 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. 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:** Time 1 (Surveys 1 & 2 sampling results): Reflects exposures before major control changes that would impact microwave quality control exposures. 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. 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 microwave production area exhaust fans; installation of a mixing room air-lock; and ventilation of mezzanine flavor holding tanks. Time 4 (Surveys 7 & 8 sampling results): Reflects the enclosure of the microwave ovens in the quality control lab. 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 I

Typical Protocol for Collecting Air Samples for Diacetyl and 2, 3-Pentanedione

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 2 - A), 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 vapors 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.

References:

Leidel NA, Busch KA [1994]. Statistical design and data analysis requirements. In: Harris RL, Cralley LV, eds. Patty's industrial hygiene and toxicology. 3rd ed. Volume 3, Part A. New York, NY: John Wiley and Sons, Inc., pp. 453–582

NIOSH [1977]. Occupational exposure sampling strategy manual. Cincinnati, OH: U.S. Department of Health, Education, and Welfare, Center for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHEW (NIOSH) Publication No. 77–173.

NIOSH [1995]. NIOSH criteria for a recommended standard: occupational exposure to respirable coal mine dust. Cincinnati, OH: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 95–106.

This page intentionally left blank.

NIOSH

Delivering on the Nation's promise: safety and health at work for all people through research and prevention

To receive NIOSH documents or more information about occupational safety and health topics, contact NIOSH at

1–800–CDC–INFO (1–800–232–4636)
TTY: 1–888–232–6348
E-mail: cdcinfo@cdc.gov

or visit the NIOSH website at **www.cdc.gov/niosh**

For a monthly update on news at NIOSH, subscribe to *NIOSH eNews* by visiting **www.cdc.gov/niosh/eNews**

DHHS (NIOSH) Publication No. 2016–111